# NEUROPSYCHOPHARMACOLOGICAL EVALUATION OF KALYANAKAM KASHAYAM (AN AYURVEDIC FORMULATION) IN SWISS ALBINO MICE


*Dissertation Submitted to*

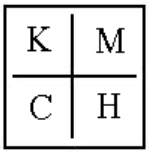
*The Tamil Nadu Dr. M.G.R. Medical University, Chennai*

*In partial fulfillment for the award of the Degree of*

**MASTER OF PHARMACY**

**(Pharmacology)**

**OCTOBER-2016**




**DEPARTMENT OF PHARMACOLOGY**

**KMCH COLLEGE OF PHARMACY**

**KOVAI ESTATE, KALAPPATTI ROAD,**

**COIMBATORE-641048**

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# CERTIFICATE

This is to certify that the dissertation work entitled "**Neuropsychopharmacological evaluation of Kalyanakam kashayam (an ayurvedic formulation) in Swiss Albino mice"** is a bonafide research work carried out by the candidate **(Reg No: 261425818)** and submitted to The Tamil Nadu Dr. M.G.R Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology** at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2015-2016.

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**Date:**

**Guide**
**Department of pharmacology**

# DECLARATION

I do hereby declare that the dissertation work entitled **"Neuropsychopharmacological evaluation of Kalyanakam kashayam (an ayurvedic formulation) in Swiss Albino mice"** submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy in Pharmacology**, was done by me at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu during the academic year 2015-2016.

**Date:** **Reg No: 261425818**

# EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled **"Neuropsychopharmacological evaluation of Kalyanakam kashayam (an ayurvedic formulation) in Swiss Albino mice"** submitted by the candidate **(Reg no: 261425818)** to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the Degree of **Master of Pharmacy** in **Pharmacology** is a bonafide work carried out by the candidate at the Department of Pharmacology, KMCH College of Pharmacy, Coimbatore, Tamil Nadu and was evaluated by us during the academic year 2015-2016.

**Date:**

**Internal Examiner** **External Examiner**

**Convener of Examinations**

**Examination Centre: KMCH College of Pharmacy, Coimbatore.**

# LIST OF ABBREVIATIONS

| SL NO | ABBREVIATIONS | FULL FORM |
|---|---|---|
| 1 | ABTS | 2, 2, - azinobis (3-ethylbenzoline-6-sulfonic acid) |
| 2 | ANOVA | Analysis of Variance |
| 3 | APM | Apomorphine |
| 4 | APOE | Apolipoprotein E |
| 5 | APP | Amyloid Precursor Protein |
| 6 | BPAD | Bipolar Affective Disorder |
| 7 | cAMP | Cyclic Adenosine Mono Phosphate |
| 8 | CNS | Central Nervous System |
| 9 | COMT | Catechol-O-Methyl Transferase |
| 10 | CPCSEA | Committee for the Purpose of Control and Supervision of Experimental Animals |
| 11 | DA | Dopamine |
| 12 | DAG | Diacylglycerol |
| 13 | DOPA | Dihydroxyphenylalanine |
| 14 | EPM | Elevated Plus Maze |
| 15 | FAD | Familial Alzheimer's Disease |
| 16 | FC | Folin Ciocalteu reagent |
| 17 | FRAP | Ferric Reducing Anti-oxidant Power |
| 18 | FST | Forced Swim Test |
| 19 | GABA | Gamma Aminobutyric Acid |
| 20 | HP | Haloperidol |
| 21 | 5-HT | 5- Hydroxytryptamine |
| 22 | IAEC | Institutional Animal Ethical Committee |
| 23 | i.p | Intraperitonial |
| 24 | $IP_3$ | Inositol Triphosphate |

| | | |
|---|---|---|
| 25 | Kg | Kilogram |
| 26 | MAO-A | Monoamine Oxidases Type-A |
| 27 | MAO-B | Monoamine Oxidases Type-B |
| 28 | MAOIs | Monoamine Oxidases Inhibitors |
| 29 | MDD | Major Depressive Disorder |
| 30 | MPTP | 1-Methyl-4-Phenyl-1, 2, 3, 6-Tetra-Hydropyridine |
| 31 | NMDA | N-Methyl D- Aspartate |
| 32 | N | Number of Animals |
| 33 | ns | non significant |
| 34 | OECD | Organisation for Economic Corporation and Development |
| 35 | P | Probability |
| 36 | PB | Pentobarbitone |
| 37 | PD | Parkinson ’s disease |
| 38 | PHF | Poly Herbal Formulation |
| 39 | p. o | Post Oral |
| 40 | PSEN | Presenilin |
| 41 | PTZ | Pentylenetetrazole |
| 42 | rpm | Rotations Per Minute |
| 43 | SEM | Standard Error Mean |
| 44 | SNARI | Selective Noradrenaline Reuptake Inhibitors |
| 45 | SNRI | Selective 5HT-NA Reuptake Inhibitors |
| 46 | SSRI | Selective Serotonin Reuptake Inhibitors |
| 47 | TPTZ | 2, 4, 6, - tripyridyl-5-triazine |

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# ACKNOWLEDGEMENT

*My dissertation entitled "**NEUROPSYCHOPHARMACOLOGICAL EVALUATION OF KALYANAKAM KASHAYAM (AN AYURVEDIC FORMULATION) IN SWISS ALBINO MICE"** would not have beena feasible one without the grace of God almighty who gave me moral till the completion of my project.*

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Reg No: 261425818

# ABSTRACT

This study was designed to evaluate the neuropsychopharmacological profile of Kalyanakam Kashayam, an Ayurvedic formulation in various experimental models. The formulation was evaluated for its anti-depressant activity using Pentobarbitone – induced sleeping time, Actophotometer test and Forced swim test. It was found out that the formulation produced significant potentiation of sleeping time induced by pentobarbitone, mild reduction in immobility time in forced swim test and significant increase in locomotor activity. Anxiolytic activity was assessed using the Elevated plus maze model and Hole board test. The formulation significantly increased the time spent by the animal in open arm without affecting the number of entries to both the arms in elevated plus maze model and a promising reduction in the number of nose poking in hole board test. The results of the study provided evidences that Kalyanakam Kashayama is a promising antidepressant, anxiolytic and antipsychotic agent. The formulation showed no significant effect on fall off time in Rotarod test which indicated the Non – neurotoxic behavior of the Formulation. In Haloperidol – induced catalepsy model, a significant reduction in cataleptic time was observed. The current study also recommended that the formulation is an impending atypical antipsychotic agent against schizophrenia using Apomorphine–induced stereotypy model and an effective anti-convulsant drug in Pentylenetetrazol – induced convulsion models.



Key words: Kalyanakam kashayam; Formulation; mice; Neuropsychopharmacological profile.

# 1. INTRODUCTION

## Neuropsychopharmacology

Neuropsychopharmacology may be defined as the branch of inter disciplinary neuroscience devoted to the study of drugs that have an effect on the nervous tissue and alter the behavior. Neuropharmacology deals with the study of effects of the drug on nerve cells, their synapses and circuit whereas the study of effects of drugs on behaviors, including emotional and cognitive mental activities, is Psychopharmacology. It associates the frontiers of fundamental neuroscience to the management of psychiatric and neurological diseases. This branch of science seeks to comprehend how drugs selectively affect the CNS to induce sleep, relieve pain, reduce fever, suppress muddled movement, prevent seizures or enhance attention. Neuropsychopharmacology seeks to understand how drugs can treat mania, anxiety, schizophrenia or depression without disturbing the consciousness. This field seeks to uncover the biological basis for intricate mental states. The goal of this field is not only to understand the nature of the alterations in biology which direct to distorted emotions and thought processes, but also to develop therapeutically priceless specific molecules which regulate the specific biologic underpinnings- namely, the as yet vague sequences of multineuronal interactions by which the behaviors emerge [1].

Advance in modern science and technology have contributed to an enormous development in the quality of human life. However, modern life stresses are responsible for the surge in incidence of variety of psychiatric disorder. Drugs that are currently used in managing different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, Parkinsonism either have severe side effects or posses inauspicious drug-drug/drug-food interactions. Moreover, the western system of medicine pays no heed to the fundamental problems and depends on drug treatment to cure the symptoms. The western restorative treatment fails to tackle the huge diversity among patients. In this context, Ayurvedic treatment, which considers a patient's entire body-mind-spirit relationship and bioindividuality which aims to look at the root cause of the disease and it's relation with the lifestyle, thoughts and beliefs of

the person (vital energy of the patient), have attained widespread fame in treating the neurologic disorders than the western system of medicine [2].

Ayurveda (Ayur = life, Veda = Knowledge) is concerned about the absolute wellness together with, psychological, physical, social wellness, economical as well as spiritual security. The prime goal of Ayurvedic medication is to facilitate people live long hence known as the science of longetivity), hale and hearty and balanced lives with no need for problematical surgery, chemical drugs or tormented through painful conditions. The basic principle of Ayurveda is the belief that distress and ailment results from disparity in three doshas (Vata, Pitta and Kapha) which are the three basic energy types of the body. These doshas are the prime determinants of body type, energy levels, appetite, tendencies and moods and are considered and checked by the physicians to prescribe the essential medication. The major aspects of Ayurvedic restoration of balance includes tuning of the natural rhythms of our body and also synchronizing our body with nature and its cyclic patterns which helps to relieve stress and also restore the circadian rhythm of our body.

The Indian subcontinent is an immense warehouse of curative plants that are used in customary medical healing. The unconventional medicines in the deep-rooted system are derivatives of herbs, organic matter and minerals, whilst for the preparation of herbal drugs only medicinal plants are used. Utilization of plants as a foundation of medicine has been a primordial practice and is a significant component of the Indian health care system. In India, approximately 70 percentage of rustic population depends upon the customary Ayurvedic scheme of medicine. Most practitioners/ healers of the conventional system of medication prepare formulations by their individual recipes and dole out to the patients. About 40% of people in the Western countries make use of the herbal medication for the management of a variety of diseases. This concern in traditional remedy is budding swiftly due to the concern being given to it by the governmental organization and various NGO's encompassing researchers and common public as well as the amplified adverse drug reactions, side effects and cost of the recent drugs.

In India, about 20,000 curative plants have been documented; nevertheless, time-honored practitioners make use of only 7,000–7,500 plants for treating diverse diseases. The ratio of use of plants in various Indian systems of remedy is Ayurveda-2000,

Siddha-1300, Unani-1000, Homeopathy-800, Tibetan-500, current-200, and folk 4500. Roughly 25,000 valuable plant-based formulations are made use of in conventional and folk medication. Over 1.5 million practitioners are using the long-established therapeutic scheme for wellbeing in India. It is anticipated that over 7800 manufacturing units are concerned with the formulation of natural health products and conventional plant-based formulations in India, which makes use of approximately 2000 tons of medicinal crude plant material per annum. About1500 herbals are vended as nutritional add-on or ethnic conventional medicines. Alternative medications are being used by people who cannot be facilitated by traditional medicinal scheme.

The breakthrough of herbals is additionally harmonized with acquaintance on the technique of seclusion, purification, portrayal of active components and category of preparation. The phrase "herbal drug" resolve the fraction/fractions of a plant (flowers, leaves, seeds, barks, roots and stems etc.) used for preparing medication. Each and every fraction of the herbs are entirely made use of for the dissimilar pharmacological action they probably will engender and refined into an array of herbal preparations together with Phanta (Hot infusion), Kwatha (Decoction), Hima(Cold infusion), Guggul (Resins and balsams), Churna (Powders), Arka (Liquid Extract), Taila (Medicated oil) and etc.

Owing to the technical or scientific encroachment today, auxiliary pharmacologically dynamic ingredients of the Ayurvedic medication with their utility in drug treatment have been recognized. Fundamentally, it is the phytochemical component in the herbs which direct to the preferred therapeutic effect, such as tannins, saponins, alkaloids, flavonoids, alkenyl phenols, terpenoids, sesquiterpenes lactones and phorbol esters. A solitary herb may yet contain more than one of the aforesaid phytochemical ingredients, which acts synergistically with each other in fabricating pharmacological action.

## Single herbals and Polyherbal formulation

Ayurvedic drug formulation is based on two ideologies; Utilized as a sole drug and use of more than single drugs, in which the latter is known as Polyherbal Formulation (PHF). This key conventional curative herbal approach takes advantage of combining of numerous therapeutic herbs to accomplish additional therapeutic efficacy, typically known as polyherbalism or polypharmacy.

In times gone by, the Ayurvedic prose "Sarangdhar Samhita" dated centuries in the past in 1300 A. D. has highlighted the perception of polyherbalism in this prehistoric curative system. In the traditional system of Indian medicine, plant formulations and combined extracts of plants are chosen rather than individual ones. Ayurvedic herbals are prepared in various types of dosage forms, in which typically almost all of them are PHF.

Even though the dynamic phytochemical components of each plant have been well recognized, they more often than not present in infinitesimal amount and constantly, they are inadequate to accomplish the enviable therapeutic effects. For this, methodical or scientific studies have exposed that these flora of changeable effectiveness when united may tentatively fabricate a superior result, as weighed against individual use of the herbals and also the totting up of their individual outcome. This trend of constructive herb-herb interaction is recognized as synergism. Some of the pharmacological actions of dynamic elements of herbals are noteworthy only when potentiated by that of supplementary plants, but not apparent when used alone.

Depending upon the nature of the interaction, synergism acts by two mechanisms (i.e., pharmacokinetic and pharmacodynamics). The capacity of herb to facilitate the absorption, distribution, metabolism and elimination of the additional herbs are focused in pharmacokinetic synergism. On the other hand, Pharmacodynamic synergism deals with the synergistic outcome when dynamic ingredients with alike remedial activity are embattled to a analogous receptor or physiological system. As a result of synergism, polyherbalism bestow some reimbursement not accessible in sole herbal formulation. It is apparent that superior restorative result can be arrived at with a solo multi-component formulation. In favor of this, an inferior dose of the herbal formulation would be desired to accomplish enviable pharmacological action, thus tumbling the peril of lethal side-effects. Moreover, PHFs fetch to enhanced expediency for patients by eradicating the need of taking additional different single herbal formulation at an instance, which ultimately guide to improved acquiescence and restorative effect. When weighed against single herbal formulations, all these reimbursement have paved way to the popularity of PHF in the market.

During the formulation of polyherbal preparations, it is decisive to note down that herbs are every so often considered to be unable to coexist (viruddha) and thus ought

not to be taken in concert. Such incongruity may be owing to quantitative inaptness, functional incompatibility or energetic incompatibility. To guarantee compatibility of manifold herbs in the PHF formulation, there is a need of ingenious clinical trials preceding marketing.

**Reasons for using PHF**

It is well-known that PHF have started to achieve its fame recently globally, due to the verity that PHFs own some advantages which is inaccessible in allopathic drugs.

At the outset, PHFs are recognized to articulate sky-scraping efficacy in an enormous number of ailments. As aforesaid, the remedial effects of herbal medications are put forth due to the incidence of dissimilar phytoconstituents and the effects are additionally potentiated when well-matched herbals are put together in PHFs.

Secondly, PHFs are typically established to have extensive therapeutic assortment. Majority of them are efficient even at a very low dose and safe and sound at elevated dose, thus they include better peril to assistance ratio.

Commonly, PHFs (restricted to those properly used manufactured) result in smaller number of side effects as weighed against allopathic drugs. While contemporary allopathic drugs are intended for effectual restorative results, management of majority of them come up with unnecessary side-effects, such as vomiting, insomnia, fatigue, diarrhea, dry mouth, seizures, confusion, impotency, organ toxicities, hair loss and even demise.

Due to the verity that PHFs are a product of the nature, they are comparatively cheaper, biodegradable and readily accessible than allopathic drugs. Their better affordability and greater accessibility explains the ever-increasing demand worldwide, chiefly in rustic areas and some developing countries, where expensive contemporary treatments are inaccessible. Furthermore, right through the history, polyherbal cure have long stand as conventional attitudes, customs and practices in certain ethnic group, which are based upon centuries old knowledge of tryout and slip-up.

All the above mentioned motives: Effectiveness, cheap, safety, better acceptance and ubiquity, made PHF the ultimate choice of treatment, for this reason superior acquiescence by the patients and exceptional curative effect is made certain.

## Major problems related to PHF usage

Regardless of the verity that Ayurvedic PHFs are valuable to mankind in loads of facet, they are still disputed by some inevitable shortcomings, upsetting their capability and effectiveness in cure. These troubles lie within the PHFs' resource and manufacturing procedure, Ayurvedic practitioners, patients, as well as the regulations and law.

There is a sturdy fallacy that Ayurvedic PHFs are constantly safe, which is fictitious. Charaka Samhita itself has depicted that Ayurvedic medications have unpleasant effects when used or prepared improperly. The concomitant use of allopathic drugs with PHFs is growing as a good number of the individual patients do not notify their medical practitioners on the parallel treatments. Nevertheless, countless have not noted the probable drug-herb interactions, which may have an effect on their pharmacological or toxicological effects, consequently results in unpleasant effects that depreciate wellbeing [3].

Herbalists consider that it is significant to confer not merely the "hardware" of the brain and nervous system, but its "software," the emotions and mind as well. Every single one of these chemical responses and reactions are indispensable to our indulgent of the nervous system, and focusing on neurotransmitters and hormones can undoubtedly bid us insight into psychological processes [4]. Mental factors apparently are concerned in nervous system wellbeing. Current proceedings in the field of neurology have come about through the assessment of allege for herbal medications. Majority of the quarter of apprehension of neurology will potentially get assistance from herbal remedies, and undeniably the discipline of psycho-pharmacology itself is principally based upon the chemicals discovered in plants [5].

In the Ayurvedic system of medication, for CNS disorders polyherbal formulations are recurrently prescribed and the plant constituent in these formulations augment the activity of compounds or neutralize the noxious effect of compounds from other plants but also work synergistically with additional constituents from the same plants. Till date no research work has been carried out on the formulation. For that reason, it was considered valuable to investigate neuropharmacological effects of Kalyanakam kashayam to corroborate the claim made by conventional healers as well as in Ayurveda.

Thus the prime concern of the study is to evaluate the nootropic activity of the selected Ayurvedic Polyherbal Formulation "Kalyanakam Kashayam" in various animal models.

## Neurotransmission

Neurotransmission may be defined as the process of transfer of impulses between neurons and Neurotransmitters are those biochemical substances responsible for the transmission of nerve signals across a synapse between two neurons.

The Central Nervous System uses a wide variety of Neurotransmitters including both Excitatory and Inhibitory chemical transmitters including:

1. Biogenic Amines
    - Dopamine
    - Norepinephrine
    - Epinephrine
    - Serotonine
    - Histamine

2. Amino Acids
    - Glutamic Acid
    - Aspartic Acid
    - Gama Amino Butyric Acid
    - Glycine

3. Peptides
    - Angiotensin
    - Endorphins
    - Vasopressin
    - Substance P etc.

4. Others
    - Purines (Adenosine & Adenosine Triphosphate)
    - Nitric Oxide

Acetylcholine and Monoamines are ought to perform specialized regulating functions, often limited to explicit structures. The peptides regulate neuronal function by themselves or in concert with other neurotransmitters and execute specialized functions mainly in hypothalamus.

Table-1: Some Neurotransmitters found in Nervous system

| Name | Effects | Receptor subtype | Receptor motif | Mechanism |
|---|---|---|---|---|
| **AMINES** | | | | |
| Acetylcholine | Both excitatory & inhibitory | Nicotinic | Ionotropic | ↑ $Na^+$, $K^+$,$Ca^{2+}$ conductance |
| | | Muscarinic | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |
| | | | | ↓ cAMP, ↑ $K^+$ conductance |
| Norepinephrine | Both excitation & inhibition in the brain stem | $\alpha_1$ | Metabotropic | ↑ $IP_3$/DAG/$Ca^{2+}$ |
| | | $\alpha_2$ | Metabotropic | ↓ cAMP,↑ $K^+$,$Ca^{2+}$conductance |
| | | $\beta_1$ , $\beta_2$, $\beta_3$ | Metabotropic | ↑ cAMP |
| Dopamine | Predominantly Inhibitory | $D_1$, $D_5$ | Metabotropic | ↑ cAMP |
| | | $D_2$, $D_3$, $D_4$ | Metabotropic | ↓ cAMP,↑ $K^+$, $Ca^{2+}$conductance ↓ |
| Serotonin (5HT) | Both excitatory & inhibitory | $5HT_1$ | Metabotropic | ↓ cAMP, ↑ $K^+$ conductance<br>↑ $IP_3$/DAG/$Ca^{2+}$ |
| | | $5HT_2$ | Metabotropic | ↑ $Na^+$, $K^+$,$Ca^{2+}$ conductance |
| | Mainly | $5HT_3$ | Ionotropic | ↑ cAMP |

| | | | | |
|---|---|---|---|---|
| Histamine | inhibitory. Functions uncertain. | $5HT_{4-7}$<br><br>$H_1$<br>$H_2$<br>$H_3$ | Metabotropic<br><br>Metabotropic<br>Metabotropic<br>Unknown | ↑ $IP_3/DAG/Ca^{2+}$<br>↑ cAMP<br>Unknown |
| Amino Acids<br>Glycine | Inhibitory | α ,β subunits | Ionotropic | ↑ $Cl^-$ conductance |
| Glutamate & Aspartate | Excitatory | AMPA<br>Kainate<br>NMDA<br>mGlu(1-7) | Ionotropic<br>Ionotropic<br>Ionotropic<br>Metabotropic | ↑ $Na^+$, $K^+$ conductance<br>↑ $Na^+$, $K^+$ conductance<br>↑ $Na^+$, $K^+$,$Ca^{2+}$ conductance<br>↓ cAMP<br>↑ $IP_3/DAG/Ca^{2+}$ |
| Gama-aminobutyric acid (GABA) | Inhibitory | $GABA_A$<br>$GABA_B$ | Ionotropic<br>Metabotropic | ↓ cAMP<br>↑ $Cl^-$ , $K^+$ conductance |

## MAJOR NEUROLOGIC DISORDERS

Human beings accomplish competence through multifaceted integrated processes. The major neural system which account for this competence includes; sensory, motor and cognition systems. Alteration in all of this or any one of them has an effect on competence. Some of the disorders associated with disturbances in the activity of neurological system include:

- Parkinson's disease
- Alzheimer's disease
- Anxiety
- Depression
- Schizophrenia [6] [7] [8]

## PARKINSON'S DISEASE

It is a neurodegenerative disorder of corpus striatum (basal ganglia) involving the dopamine-secreting nigrosriatal pathway. There is a profound loss of dopaminergic neuron (atleast 70%) when the first symptoms appear. The onset of the ailment occurs following 40 years of age. Men are more prone to the disease than women. It is one of the most prevalent neurological disorders in individuals older than 60 years. Approximately 107 to 187 per 100,000 persons are affected by the disorder.

### Etiologic classification

**Primary Parkinson's disease**- Loss of pigmented neurons in the substantia nigra, chiefly in the medial and ventral segment allied with reactive gliosis.

**Secondary Parkinson's disease**- Caused by disorders like infection, trauma, neoplasm, toxins, atherosclerosis, drug intoxication etc. Drugs like neuroleptics, anti-hypertensives, anti-emetics etc are the common cause of drug induced Parkinsonism. 1-methyl-4-phenyl- 1, 2, 3, 6-Tetrahydropyridine (MPTP) produces a Parkinsonian syndrome due to the degeneration of locus ceruleus and substatia nigra, in users.

### Pathophysiology

The pathogenesis of the disease is not evidently known. Studies illustrate that there is an autosomal dominant form involving mutation in alpha synuclein gene on Chromosome 4 (4q21.23) and an autosomal recessive form (ARDP) involving mutation in the parkin gene on Chromosome 6 (6q 25.2-27).

## Pathologic feature:

Dopaminergic nigrostrial pathway composed of 'Black substance' (neurons of substantia nigra) with the fibres synapsing in the caudate and putamen basal ganglia. Exhaustion of dopamine in substatia nigra and basal ganglia is the prime biochemical alteration in Parkinsonism. In the disease, depletion of dopamine in the basal ganglia and a comparative excess of cholinergic activity in feedback circuit linking basal ganglia, thalamus and cerebral cortex, occurs as a result of nigrostraital dopaminergic pathway. Age influences nigrostriatal by environmental toxins. MPTP (chemical neurotoxin) have the capacity to selectively destruct the cells of substantia nigra and and can produce irreversible Parkinsonian syndrome.

## Clinical Manifestations

- Resting tremor involving 'pill-rolling' movements of the hand.
- Slow initiation of movements (Bradykinesia)
- Abnormal posture
- Muscular rigidity
- A blank facial expression
- A characteristic shuffling gait
- Inability to perform skilled tasks.
- Speech impairment.

## Drug therapy

**The drug therapy for Parkinson's disease includes**:

1. Drugs increasing Dopaminergic activity
    - Precursors of Dopamine: Levodopa
    - Inhibitors of Dopamine metabolism:
        - ❖ COMT inhibitors: Tolcapone, entacapone
        - ❖ MAO-B inhibitors: Selegiline,rasagiline
    - Dopamine releasers: Amantadine
    - Dopamine receptor agonists:

- ❖ Ergot derivatives: Bromocriptine, Pramipexole, Ropinirole
- ❖ Non-Ergor derivatives: Lysuride

2. Drugs that affect brain cholinergic system
   - ❖ Central anti-cholinergic drugs: Procyclidine, benzhexol
   - ❖ Antihistaminics: Promethazine, orphenadrine [9] 10] [11] [12] [13].

## SCHIZOPHRENIA

Schizophrenia may be defined as a thought disorder typified by one or more episodes of psychosis i.e. impairment in reality testing. Individuals in their late teens and early 20's are affected by schizophrenia. Schizophrenia is ought to have a multifactorial etiology, involving both environmental and genetic components.

## Pathogenesis

The pathogenesis of schizophrenia is best explained by the Dopamine hypothesis. The hypothesis states the disorder is caused as a result of the deregulated and increased brain Dopamine levels. The deregulation is proposed to occur at meticulous anatomic locations in brain. Mesolimbic system is such a system which is believed to be the root cause of the positive symptoms of schizophrenia (due to hyperactivity of the system), whereas the dysfunction of alterations in the Mesocortical system is responsible for the negative symptoms of the disease.

The dopamine hypothesis is supported by fact that some $D_2$ antagonistic drugs relieves the symptoms of the disorder, while CNS dopamine receptor activators like cocaine, apomorphine etc worsens the disorder which is subsided when the dose of the drugs are reduced.

It was also observed that, Schizophrenia like symptoms are aggravated in some patients taking phencyclidine (NMDA receptor antagonist) which suggests the role of imbalance in glutamate neurotransmission in schizophrenia.

## Symptoms:

### 1. Positive symptoms

- Hallucinations
  - Visual
  - Auditory
  - Somatic- tactile
- Delusions
  - Delusions of mind reading
  - Delusions of being controlled
  - Delusions of reference
  - Persecutory
- Positive formal thought disorder
  - Derailment
- Bizarre behavior
  - Stereotyped, repetitive
  - Agitated, aggressive

### 2. Negative symptoms

- Affective flattening
  - Decreased spontaneous movements
  - Affective non responsivity
  - Lack of vocal inflections
- Alogia
  - Poverty of speech
  - Blocking
- Anhedonia – associality
  - Few social relationships
  - Few recreational interests
- Attention
  - Inattentiveness during testing

Social inattentiveness

- Avolition – apathy

  Lack of persistence

  Physical anergia

## Drug therapy:

Antipsychotic drugs

- Phenothiazines: Chlorpromazine
- Rauwolfia alkaloids: Reserpine
- Butyrophenones: Haloperidol
- Diphenylbutylpiperidines: Pimozide
- Thioxanthenes: Flupentixol
- Indole derivates: Molindone
- Dibenzodiazepines: Clozapines
- Substituted benzamides: Sulpiride
- Miscellaneous: Olanzapine [14] [15] [16].

## ALZHEIMER'S DISEASE

It is a common neurologic disorder which is also known as senile disease complex or dementia of Alzheimer's type. It is a common disease of elderly people and is found to be the major severe cognitive dysfunction. The major risk factor of the disease is age. The prevalent forms of the disease are:

- Early-onset familial dementia
- Late-onset familial Alzheimer's disease (FAD)
- Sporadic or nonhereditary late onset Alzheimer's disease it is also associated with Down's syndrome (SDAT).

## Pathophysiology

The underlying cause of Alzheimer's disease is unknown. The possible theories of the pathogenesis of the disease include:

- Lossx of Choline acetyltransferase induced neurotransmitter stimulationt.
- Apolipoprotein –E alterations, to which beta amyloid binds.
- Mutation for encoding amyloid precursor protein.
- Excessive influx of calcium ions as a result of the pathologic activation NMDA receptor.

Early onset FAD: Caused as a result of atleast 3 gene defects; presenilin 1 (PSEN1) gene on chromosome 14, amyloid precursor protein (APP) gene on 21 and presenilin 2 (PSEN2) gene on chromosome 1.

Late onset FAD: caused due to the defective gene apolipoprotein E (APOE4) on chromosome 19.

All these mechanisms may result in the aggregation and precipitation of amyloid which is insoluble in nature in brain tissues and blood vessels. The disease is linked to a lysosomal pathway which yields beta amyloid (neurotoxic) as a result of the breakdown of amyloid precursor protein. Alzheimer's disease has an autoimmune cause as well as the presence of antibrain antibodies.

Another hypothesis regarding the disease is the attachment of complement proteins to the formed plaques leading to the microglial cell attraction to the plaques. These cells release toxins inorder to destroy the plaques which predispose to the disease. The disease development depends upon the changes that occur as a result of ageing and injury.

### Clinical manifestations

- Forgetfulness and Emotional upset
- Disorientation and Confusions
- Inability to concentrate

- Problem solving and abstraction declines
- Lack of judgment etc [17] [18] [19].

## AFFECTIVE DISORDERS

They are exemplified by mood regulation. The monoamine hypothesis proposes that, the mood disorders are caused due to the reduced norepinepinephrine and /or serotonin levels. It is also suggested that these disorders reflect complex disturbances rather than simple chemical imbalance. The major affective disorders include;

1. Major depressive disorder
2. Bipolar Affective Disorder

## 1. Major depressive disorder (MDD)

Characterized by recurrent episodes of social isolation (including decreased ability to experience pleasure, apathy, feelings of worthlessness), depressed mood, and somatic symptoms (alterations in sleep and appetite, reduced energy, speech latency, muscle pain and slowing of movement. It is precipitated by major life stresses or occurs spontaneously. A single depressive episode is believed to last for 2 weeks or longer and to interfere with the patient's day to day activity.

**Subtypes of MDD**:

- Melancholic depression (typical depression)
- Psychotic depression
- Atypical depression

**Melancholic depression** (typical depression)

Characterized by the inability to go back to sleep after waking up early in the morning, prominent social detachment etc.

**Atypical depression**

It is characterized by symptoms opposite to the signs shown by patients with typical depression. They will exhibit increased appetite with hypersomnia. They are capable of enjoying brief period's pleasure and indulge in pleasure seeking activities.

**Psychotic depression**

It is the major disabling and severe form of depression.

## 2. Bipolar Affective Disorder (BPAD)

It is characterized by mood swing from hypomania to depression. Usually the dominant characteristic features of BPAD include noteworthy, incapacitating depression. Before the attack by manic episodes, usually the patient is struck by depressive episodes.

## **Drug therapy**:

### Antidepressant Drugs

1. Monoamine oxidase inhibitors (MAOI)
   - Irreversible:
     Hydrazine derivatives: Iproniazid, isocarboxazid
     Nonhydrazine derivatives: Tranylcypromine
   - Reversible: Moclobemide, Clorgyline
2. Serotonin – Noradrenaline reuptake inhibitors
   - Tricyclic Antidepressants
     NA- reuptake inhibitors: Amitryptyline
     5HT- reuptake inhibitors: Clomipramine
   - Selective 5HT-NA reuptake inhibitors (SNRI): Duloxetine, Venlafaxine
3. Selective serotonin reuptake inhibitors (SSRI): Fluoxetine, Citalopram
4. Selective Noradrenaline reuptake inhibitors (NARI): Reboxetine
5. 5-$HT_2$ receptor blockers: Trazodone
6. Miscellaneous
   Unicyclic: Bupropion

Tetracyclic: Amoxapine [20] [21] [22].

## ANXIETY DISORDERS

Anxiety may be defined as an unpleasant state of mental uneasiness, apprehension, nervousness and obsession or concern about something uncertain. The major symptoms include; arousal, tenseness, increased autonomic activity like respiration, blood pressure and heart rate. Tightness in chest, palpitations, perspirations etc. it is a common symptom in a variety of distinct mental illnesses and is a predominant symptom in panic disorders, phobias and obsessive compulsive disorder.

The major types of anxiety include:

**Panic disorder**: Psychiatric condition associated with multiple disabling panic attacks. In between the panic attacks, an excessive time is spent by the individual in thinking about future panic attacks.

**Generalized anxiety disorder**: It is characterized by persistent and excessive worries. The patient worries about various life events such as job performances, marital status, money, social status etc.

**Posttraumatic stress disorder**: Caused due to the exposure of individual to life-threatening or terrifying life events. The individual re-experiences the traumatic event as flashbacks or as intrusive recollections.

**Obsessive – compulsive disorders**: The root cause of the disorder is intrusive, repetitive compulsions and / or thoughts. Marked distress is the hallmark of this disorder. There occur irrational thoughts and acts which impair normal functioning.

## Drug therapy:

## Antianxiety Drugs:

- Benzodiazepines: Diazepam, lorazepam, alprazolam
- Azapironees: Buspirone
- Sedative antihistaminic: Hydroxyzine
- Beta blocker: Propranolol [23] [24] [25].

# 2. REVIEW OF LITERATURE

The following studies on the constituents of the Formulation were reported in literature.

- Shalini Adiga *et al*., (2010) evaluated the effect of *Punica granatum* peel extract on learning and memory in rats. At the doses used (50 and 100mg/kg), there is an explicit trend of memory advancement by *Punica granatum* peel with effects being more noticeable on spatial learning tendency and long term memory than on retention capacity at a dose of 100mg/kg [26].

- Amin Ataie *et al*., (2010) studied the Neuroprotective effects of the polyphenolic antioxidant agent, Curcumin, against homocysteine-induced cognitive impairment and oxidative stress in rat. In this study,the neuroprotective and antioxidant properties of the extract was evaluated against the neurotoxicity induced by Homocysteine. The results showed that the Intraperitonial injection of curcumin (5 and 50mg/kg) and intra-hippocampal injection of homocysteine (0.2µmol/ml) for a specified time period remarkably reduced the level of Malondialdehyde and super oxide anion (increased by homocysteine injection). The histopathological analysis indicated the ability of curcumin extract to control the reduction in hippocampus cell count, thereby reversing the toxic effect of homocysteine. Thus they concluded that the polyphenol treatment (Curcumin) showed a significant improvement in learning and memory deficits by protecting the nervous system against homocysteine toxicity [27].

- Justyna Pyrzanowska *et al*., (2010) evaluated the influence of the long-term administration of *Curcuma longa* extract on learning and spatial memory as well as the concentration of brain neurotransmitters and level of plasma corticosterone in aged 24 month old male Wistar rats using Morris water maze paradigm. The extract at doses 10 and 50mg/kg orally for 2 months showed noteworthy differences in brain monoamines and amino acid levels between groups. The result showed an enhanced learning ability and spatial memory, modulation of central serotoninergic system activity and increased tolerance to stress in animals after curcuma extract treatment. The extract showed hopeful

reduction in glutamate induced excitotoxicity and hippocampus neuronal degeneration [28].

- Hanumanthachar Joshi *et al*., (2006) studied the antiamnesic effects of *Desmodium gangeticum* to evaluate the capacity of the plant as a nootropic agent using exteroceptive behavioral models like elevated plus maze, passive avoidance paradigm and interoceptive behavioral models including Scopolamine induced amnesia and ageing induced amnesia. The treatment with the extract for seven successive days at doses (50, 100, and 200mg/kg. p. o.) reversed the amnesia induced by scopolamine (0.4mg/kg. i. p.) and natural ageing. The extract was also found to reduce the whole brain acetylcholine esterase activity [29].
- Subha Rastogi *et al*., (2011) carried out a study on the ethnomedicinal, phytochemical and pharmacological profile of *Desmodium gangeticum (L.)* DC. and *Desmodium adscendens (Sw.)* DC and concluded that Desmodium gangeticum and *Desmodium adscendens* have emerged as a good source of traditional medicine. *Desmodium gangeticum* possessed the ability to scavenge the free radicals generated during ischaemia and ischaemic reperfusion thereby preserving the mitochondrial respiratory enzymes that eventually led to cardio-protection, and is a promising antiamnesic or nootropic agent [30].
- Gaurav Gupta *et al*., (2012) evaluated the Antidepressant-like activity of Embelin isolated from *Embelia ribes*. Extracted Embelin at doses (2.5 and 5 mg/kg) was administered intraperitonially and the anti-depressant activity of the extract was evaluated using Tail suspension test and Forced swim test. The extracted Embelin at a dose of 5 mg/kg showed comparable decrease in immobility with that of the standard drug Imipramine (15 mg/kg) [31].
- Shete R V *et al*., (2010) studied the Nootropic effect of *Hemidesmus indicus* in mice. The learning and memory parameters were measured using elevated plus maze and passive avoidance paradigm. Thus from the study it was concluded that the n-butanol fraction of *H. indicus* extract significantly improved learning and memory at doses (3, 10 and 30 mg/kg p. o.). Hence useful as a memory restorative agent in the treatment of dementia seen in the Alzheimer's disease [32].

- David J. Loren *et al*., (2005) carried out a study entitled Maternal Dietary Supplementation with Pomegranate Juice Is Neuroprotective in an Animal Model of Neonatal Hypoxic-Ischemic Brain Injury. Pregnant mice were provided drinking fluid that contained either a high dose of pomegranate juice concentrate diluted in the ratio of 1:80 in deionized, filtered water estimated to provide 32 μmol of polyphenols per day, a middle dose of pomegranate juice (1:160 dilution of pomegranate juice concentrate estimated to provide 16 μmol of polyphenols per day), or a low dose of pomegranate juice (1:320 dilution of pomegranate juice concentrate estimated to provide 8 μmol of polyphenols per day). Sugar water control was prepared to replicate the sugar mixture and content of the middle dose dilution of pomegranate juice concentrate. The results revealed that pups in litters that were exposed to low-, middle-, and high-dose pomegranate juice had much less brain injury. The group that received high-dose pomegranate juice had >50% less tissue injury compared with the plain water control group, and the effect in this group was of highest statistical significance [33].
- Chitra V *et al*., (2009) studied the neroprotective activity of *Rubia cordifolia* on β-amyloid Induced Cognitive Dysfunction in Mice using various animal models. The study revealed the ability of the plant extract to reduce β- amyloid induced cognitive and memory dysfunction. It was concluded that the extract possessed significant protective effect on neurodegeneration and showed promising improvement in the memory retention activity which made the plant effective in treating Alzheimer's disease [34].
- Jiban Debnath *et al*., (2011) evaluated the Anti-stress Effects of *Terminalia chebula*. The animal models used for the study were anoxia stress tolerance and forced swimming test in mice, as well as cold resistant stress and immobilization test in rats. *Terminalia chebula* was administered at doses of 200 and 500 mg/kg and the reference standard used was *Withania somnifera*. The results showed that the ethanolic extracts of *T. chebula* appreciably increased the swim endurance and anoxia stress tolerance time and hence concluded the anti-stress activity of the plant by preventing stress induced elevated levels of biochemical and hematological changes and the variation in organ weights [35].
- Nitesh Garg *et al*., (2012) carried out the phytochemical studies and anti anxiey activity of uraria picta leaves. The study revealed the presence of phytoconstituents like flavonoids, steroids, triterpinoids and the plant showed good CNS related

pharmacological activities like antiaxiety activity at the doses of 400mg/kg and 600mg/kg. The extract was found to be a promising nootropic agent [36].

- Md. Shalam *et al*., (2009) evaluated the Neuropharmacological profile of Trans-01 a polyherbal formulation in mice. Trans-01, a polyherbal formulation consisting of *Valeriana wallichi* as a mojor constituent was explored for its CNS activity. The experimental models used for evaluating the nootropic activity were Hole board test, pentobarbitone sodium induced sleeping time and rotarod test. The formulation at 200, 400 and 600 mg/kg showed dose dependant anti-anxiety activity in hole board test and irrelevant effect on rotarod test and pentobarbitone induced sleeping time. The formulation at a dose of 800 mg/kg showed promising anxiolytic activity in hole board test, reduced motor coordination and skeletal muscle relaxant property in rotarod test [37].
- Amrendra Kumar Chaudhary *et al*., (2013) evaluated the cognitive enhancement in aged mice after chronic administration of *Cedrus deodara Loud* and *Pinus roxburghii Sarg.* The effect of oil and chloroform extracts of the plant on learning and memory was studied using the Morris water maze paradigm. Out of the oil and chloroform extracts of *Cedrus deodara*, chloroform extract (100 mg/kg) was found to reduce the level of malondialdehyde (MDA) with a simultaneous significant increase in the level of glutathione (GSH) in both the frontal cortex and hippocampus. The study revealed that owing to the strong anti-oxidant activity of the extract, the plant showed promising memory enhancing effect [38].
- Pinkesh K Tiwari *et al*., (2012) studied the anticonvulsant activity of *Mesua ferrea Linn* ethanolic flower extract in Albino mice at doses 200, 400 and 600 mg/kg p. o using Maximum Electroshock Seizure (MES) test. The results showed a dose dependant reduction in the duration of hind limb tonic extension (HLTE), which confirmed the plant to be a promising anticonvulsant [39].
- Edula Vinitha *et al*., (2014) evaluated the Neuroprotective effect of *Prunus avium* on streptozotocin induced neurotoxicity in mice. The neurobehavioral changes were studied using elevated plus maze and Y-maze models and the biochemical markers such as acetylcholinesterase (AChE), tissue nitrite, corticosterone, thiobarbituric reactive substances (TBARS) antioxidants like glutathione peroxidase (GPx), superoxide

dismutase (SOD) and catalase were estimated. The results of the study exposed the efficacy of the extract in averting neurotoxicity and significant diminution of AChE, corticosterone, TBARS, tissue nitrite levels [40].

- Singh Karam *et al*., (2012) carried out a study entitled An Ayurvedic insight towards epilepsy. The study revealed the effectiveness of selected plants like *Saussurea lappa, Rubia cordifolia, Emblica officinalis, Terminalia chebula, Hemidesmus indicus, Curcuma longa, Cedrus deodara* etc as potent anticonvulsant agent with least side effects and effective activity [41].
- Shrinivas K. Kulkarni (2007) studied the possible involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) signaling pathway in the antidepressant activity of berberine chloride. The antidepressant activity was assessed in forced-swim and tail-suspension tests. The results showed that Berberine (5-20 mg/kg, i.p.) produced a drop in immobility period in both the tests. The neurochemical analysis revealed that berberine (5 mg/kg, i.p.) augmented the levels of norepinephrine, serotonin or dopamine in the mouse whole brain and he concluded that by modulating brain biogenic amines (norepinephrine, serotonin or dopamine) Berberine also exerted antidepressant like effect in various behavioural paradigms of despair and interaction with the L-arginine-NO-cGMP pathway involved the antidepressant-like effect of berberine in the forced-swim test [42].
- Ashwani Kumar *et al*., (2013) carried out the Estrogenic and Anti-Alzheimer's studies of *Zingiber officinalis* and *Amomum subulatum Roxb*. Estrogenic effect was evaluated by uterine weight method while effect on learning and memory was studied using elevated plus maze method. The results of the study revealed that the Cardamom extract showed better estrogenic effect as compared to ginger and reverse trend was observed in case of anti-Alzheimer's activity. Both extracts exhibited considerable improvement in learning and memory [43].
- T. Nirmala *et al*., (2015) reviewed the herbs which exhibited anxiolytic activity in which over 40 medicinal plants were tested for their anxiolytic activity using animal models including Immobilization induced anxiety, elevated plus maze model, light dark model. Hole board test etc. The results showed the ability of the herbs to modulate the

neurotransmission process via neurotransmitters including GABA, serotonin, noradrenalin etc [44].

- Om Prakash Rout *et al*., (2013) carried out a critical review on the management of psychosomatic disorders through Ayurvedic drugs. A number of Ayurvedic drugs and their active principles were subjected for evaluation of their nootropic activity. It was observed that customarily almost 28 solitary drugs are being used either as a single or in combination for the treatment of Unmada, Apasmara, Sanyasa Bhrama, and Murccha. Almost 78 lone Indian curative plants and 50 conventional Ayurvedic formulations are being used for curing psychosomatic disorders. The medicinal herbs found to produce nootropic activity include *Callicarpa macrophylla, Emblica officinalis, Terminalia bellerica, Nymphea stellata, Curcuma longa* etc. Active constituents extracted from explicit parts of 14 curative plants are being used for managing mental health problems and nearly 46 medicinal plants were reported to be pharmacologically effective as anxiolytic, adaptogenic and anti-stress agents [45].
- B. Vinutha *et al*., (2007) reviewed the acetycholinesterase inhibitory activity of traditionally used medicinal plants and concluded that conventionally used medicinal plants like *Alpinia galanga, Rubia cordifolia, Embelia ribes, Emblica officinalis, Terminalia chebula, Punica granatum* etc showed significant acetylcholinesterase inhibitory activity [46].
- X. Xia *et al*., (2007) evaluated the Behavioral, neurochemical and neuroendocrine effects of the ethanolic extract from *Curcuma longa L*. in the mouse forced swimming test. The administration of ethanolic extract of *Curcuma longa* orally for 21 consecutive days resulted in a reduction in the duration of immobility in forced swim test. The results confirmed the promising anti-depressant activity of the plant [47].
- Khandelwal Vinoth Kumar Megraj *et al*., (2011) studied the biological activities of some traditional medicinal plants which include plants like *Emblica officinalis, Curcuma longa, Hemidesmus indicus*, *Adhatoda vasica, Aegle marmelos, Aloe vera, Andrographis paniculata, Asparagus adscendents, Cinnamomum tamala, Coriandrum sativum, Cuminum cyminum* etc. The results confirmed the promising nootropic activity of the plants. It was concluded that the reported activity may be shown by either the whole plant, or a part of the plant, or a particular extract, or isolated compounds [48].

- Sokindra Kumar *et al*., (2008) evaluated the protective effects of *Punica granatum* seeds extract against ageing and scopolamine induced cognitive impairments in mice. The results revealed the ability of ethanolic extract of the plant to overturn the age induced or scopolamine induced retention deficits in elevated plus maze and one trial step-down type passive avoidance [49].
- Jagdeep S. Dua *et al*., (2009) studied the role of traditional medicine in neuropsychopharmacology. The study was done using plants like *Valeriana wallichii, Curcuma longa, Terminalia chebula* etc. From the results, it was understood that the aqueous extract of plant of *Curcuma longa* (Turmeric) demonstrated activity in mice following oral administration, which was associated with the inhibition of brain monoamine oxidase A. Curcumin, the major constituent from this plant was shown to be neuroprotective against ethanol induced brain injury. The results further showed the ability of these plants to treat various neurological disorders [50].
- Talha Jawaid *et al*., (2011) reviewed the anti-depressant activity of medicinal plants like *Curcuma longa, Berberis aristata, Emblica Officinalis, Valeriana wallichi, Clitoria ternatea* etc and reported that herbal plants are very rich source of substance which are responsible for increasing the antidepressant activity. The plants used for the study exhibited promising nootropic activity [51].
- R. S. Ramsewak *et al*., (2000) evaluated the antioxidant activity of *Curcuma longa*. The results showed significant inhibition of liposome peroxidation by curcumins I-III at aconcentration of 100 µg/ml. Curcumins also exhibited promising inhibition of COX-I and COX-II enzymes at doses 125 µg/ml and 125 mg/ml respectively [52].
- Ravindra G. Mali *et al*., (2008) reviewed the phytochemistry, ethnobotany, pharmacologic profile of *Baliospermum montanum* and found out that the plant has the ability to reverse dropsy [53].
- Nabila Benariba *et al*., (2013) carried out the Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* and concluded that the plant extract at a concentration of 2000 µg/ml showed significant free radical scavenging activity by using DPPH method[54].
- Nithya Narayanaswamy *et al*., (2011) studied the anti-oxidant properties of *Solanum indicum* using DPPH radical scavenging activity. The total phenolic content of the plant

extract was also measured. The herbal extracts of the plants were prepared with the solvents namely water and ethanol at 5 % concentration levels. The results showed that the plant extract exhibited the DPPH radical scavenging competence ranging from 70-90 % in aqueous solvent at this particular concentration [55].

- Bibhabasu Hazra *et al*., (2010) carried out a comparative study on the anti-oxidant potential of the plants *Terminalia chebula, Terminalia bellerica* and *Emblica officinalis* and concluded that the free radical scavenging activity followed the order *Terminalia chebula* showing most promising scavenging activity followed by *Emblica officinalis* and then *Terminalia bellerica*. The flavanoid content of the plant also followed the same order but the plant containing highest concentration of phenol was found to be *Emblica officinalis* followed by *Terminalia bellerica* and finally *Terminalia chebula*. The study revealed the fact that 70% methanolic extract of the fruits of the 3 plants showed significant anti-oxidant potential [56].
- Mariel Marder *et al*., (2003) studied the CNS activity of Valeriana flavanoids obtained from various Valeriana species. Sodium thiopental-induced sleeping-time assay, Locomotor activity assay, Holeboard test, Horizontal wire test and Elevated plus-maze test were used to assess the activity of the plant. The results of the study revealed that the plant possess significant nootropic activity [57].
- Obed Ahmed Ansari *et al*., (2013) studied the anti-dementing activity of Saraswata ghrita, a nootropic formulation from Ayurveda, and concluded that the formulation was found to manage dementia in Alzheimer's disease [58].
- Gaurav Gupta *et al*., (2012) evaluated the Sedative, antiepileptic and antipsychotic effects of *Viscum album L*. (Loranthaceae) in mice and rats. PTZ-induced convulsion, Pentobarbital sleeping time and Apomorphine-induced stereotypy were the models used to assess the activity. The results suggested that the plant exhibited promising sedative, antiepileptic and antipsychotic activity in mice and rats [59].
- F. Cı´cero Bezerra Felipe *et al*., (2007) studied the anxiolytic and anti-depressant activity of Piplartine using Rota rod, elevated plus maze, pentylenetetrazole (PTZ)-induced seizures, forced swimming tests and open field test as animal model. The results revealed the promising anxiolytic and anti-depressant activity of the plant extract containing the

alkaloid piplartine which makes it an effective agent for treating anxiety and depression [60].

- Girish S. Achliya *et al*., (2004) evaluated the anti convulsant and sedative properties of the formulation Unmadnashak Ghrita using animal models including motor coordination test, locomotor activity test, PTZ- induced convulsion, Pentobarbital induced sleeping time etc. The results of the study revealed that the formulation showed CNS-depressant activity in gross behavioural test, potentiated pentobarbitone sleeping time and there was significant decrease in spontaneous locomotor count in mice. The formulation also antagonized the behavioral effects of CNS-stimulant drug amphetamine, and showed analgesic effect in mice. UG failed to affect the motor coordination test. The formulation also protected mice from MES and PTZ induced convulsions. These results suggest that UG has CNS-depressant and anticonvulsant activity in mice. [61].
- Biyani D. M *et al*., (2013) studied the CNS activity of Asthamangal Ghrita which is an Ayurvedic formulation made up of ghee (base) along with *Hemidesmus indicus,* Rock salt, *Acorus calamus, Centella asiatica, Brassica campestris, Saussurea lappa* and *Piper longum*. The activity was evaluated using various animal models which revealed the efficacy of the formulation as a promising nootropic agent [62].

# 3. FORMULATION PROFILE

## 3. 1. Name

Kalyanakam Kashayam

## 3. 2. Description

Concentrated decoction prepared out of herbal ingredients, which consist of water soluble active principles.

## 3. 3. Indication

Neurological disorders, epilepsy, psychosis and anxiety.

## 3. 4. Dose

Kashayam 5 – 15 ml diluted with 15 – 45 ml of water twice daily before food or as directed by physician.

## 3. 5. Side effects

No known side effects, over dose may produce gastric irritation [63].

## 3. 6. Ingredients

| Table-2: Composition of Kalyanakam Kashayam | | |
|---|---|---|
| Sl no. | **Sanskrit Name** | **Botanical Name** |
| 1 | Haritaki | *Terminalia chebula* |
| 2 | Vibhitaki | *Terminalia bellirica* |
| 3 | Amalaki | *Emblica officinalis* |
| 4 | Vishala | *Citrulus cholocynthis* |
| 5 | Bhadra ela | *Amomum subulatum* |
| 6 | Devadaru | *Cedrus deodara* |
| 7 | Elavaluka | *Prunus avium* |
| 8 | Sariva | *Hemidesmus indicus* |
| 9 | Haridra | *Curcuma longa* |
| 10 | Daruharidra | *Berberis aristata* |
| 11 | Shalaparni | *Desmodium gangeticum* |
| 12 | Prishnaparni | *Uraria picta* |
| 13 | Phalini | *Callicarpa macrophylla* |
| 14 | Nata | *Valeriana wallichi* |
| 15 | Brihati | *Solanum indicum* |
| 16 | Kushta | *Saussurea lappa* |
| 17 | Manjishta | *Rubia cordifolia* |
| 18 | Nagakesara | *Mesua ferrea* |
| 19 | Dadimaphalatwak | *Punica granatum* |
| 20 | Agaru | *Alpinia galanga* |
| 21 | Vella | *Embelia ribes* |
| 22 | Talisapatra | *Abbies webbiana* |
| 23 | Ela | *Elettaria cardamomum* |
| 24 | Malati | *Jasminum sambac* |
| 25 | Utpala | *Nymphea stellata* |
| 26 | Danti | *Baliospermum montanum* |
| 27 | Padmaka | *Prunus poddum* |

## 3. 7. Description of individual ingredients

- *Terminalia chebula* and *Terminalia bellirica*: The plant consists of Chebulin, a coumarin conjugated with gallic acid, chebulosides I and II, arjungenin, arjunglucoside I, punicalagin, ellagic acid, chebulinic acid, gallic acid, ethyl gallate etc and is used as laxative, purgative, appetite stimulant, rejuvenate, astringent, asthma, etc and fruits play an imperative role in endorsing memory and intelligence [64].
- *Emblica officinalis*: Chief chemical components are emblicanin A&B, pedunculagin, Vitamin C, puniglucanin, glutamic acid, aspartic acid, proline, alanine, ellagic acid hexahydroxy-diphenic acid and lysine. The activities of the plant includes antipyretic, analgesic, anti-tussive, anti-atherogenic, adaptogenic, cardioprotective, gastroprotective, anti-anaemia, wound healing, anti-hypercholesterolemia, anti-atherosclerotic, hepatoprotective, neuroprotective properties, anti-diarrhoeal and nephroprotective [65].
- *Citrulus cholocynthis*: Fatty acids such as myristic, palmitic, stearic, oleic, linoleic and Linolenic acid, Flavone c-glucosides, iso-vitexin, iso-orientin and iso-orientin 3'-methylether, while the aerial parts contain three C-p-hydroxy benzyl derivatives viz., 8-C-p-hydroxybenzylisovitexin, 6-C-p-hydroxybenzylvitexin and 8-C-p hydroxybenzylisovitexin 4'-O-glucoside are the major constituents of the plant. It was reported to possess activities like purgative, anti-diabetic, anti-inflammatory, analgesic, abortifacient, hair growth-promoting and anti-epileptic. It is combined with several other cathartics to relieve health troubles such as obstinate edema, amenorrhea, jaundice, worm infestation, bronchitis, abdominal disorders, asthma, and cerebral derangements [66].
- *Amomum subulatum*: The uses of the plant are; relief of nausea, particularly morning sickness in pregnancy, reinforces digestion and exterminates the bacteria accountable for bad breath. It also clears congestion from colds, allergies and flu. Cardamom was reported as the best source of a phytochemical called cineole, which calms your nerves and clears our head. It consists of monoterpenic hydrocarbons (16.3%), oxygenated monoterpenes (75.2%) and sesquiterpenes (6.3%). Its major constituents are 1,8-cineole (61.3%), α-terpineol, α- and β-pinene and allo-aromadendrene [67].

- *Prunus avium*: The plant is composed of chemical constituents including polyphenolic anthocyanin glycosides, melatonin, lutein, trace elements like copper, zinc, potassium etc. Melatonin has a soothening effect on nerves of the brain which relieves neurosis, insomnia, headache, fibromyalgia, heart diseases etc. It is a potent anti-inflammatory agent [68].
- *Hemidesmus indicus*: The plant is rich in coumarinolignoids, Hemidesminine, hemidesmin I and hemidesmin II50. It is effective as a tranquilizer, anti-inflammatory and anti-bacterial agent. It cools urinary tract, alleviates kidney disorders, skin diseases, epileptic seizures, high stress etc. improves fertility, helps in achieving easy dream lucidity during REM sleep [69].
- *Curcuma longa*: Curcumin is the principal constituent present in the plant and the plant is used as an anti-inflammatory, anti-cancerous, anti-allergic agent etc. It is used to cure heart burns, ulcerative colitis, bronchitis, headache and leprosy. It is found to be effective in treating Alzheimer's disease and is a cognitive enhancer. It is also used as an anti-depressant [70].
- *Berberis aristata*: Berberine, palmatine, columbamine are the principal alkaloids present in the plant. The reported activities of the plant includes, astringent, bitter tonic, anaesthetic, in treating eye diseases etc [71].
- *Uraria picta*: Alkaloids, glycosides, steroids, tannis, flavanoides and proteins are the major constituents of the plant. The plant is used in the treatment of delirium, psychosis, anxiety, ulcer, dysentery, asthma, bleeding piles, gonorrhea etc. heart diseases and snake bites are effectively treated by this plant [72].
- *Callicarpa macrophylla*: It is used for treating rheumatic pain, headache, stomatitis, genitourinary diseases, giddiness, emesis, leprosy,mouth and tongue sores. The activities are ought to be due to the presence of calliterpenone and its monoacetate, fatty acids, betasitosterol and its beta-D-glucoside [73].
- *Valeriana wallichi*: Chatarine and valerianine are the major constituents of the plant. The activities of the plant include anti-hypertensive, anti-stress and anti-anxiety activity. It is used to treat insomnia, nervous disorders, muscle spasms, menustral cramps, hysteria, asthma etc [74].

- *Solanum indicum*: Wax, fatty acids, alkaloid solanine and solanidine, disogenin, lanosterol, β-sitosterol, solasornine, solamargine and solasidine etc are the chief chemical constituents of the plant. It is traditionally used in the treatment of sleep disorders, body and toothaches etc. The plant is famous for its narcotic properties and is used as a tonic. It is used as digestive and stomachic too [75].
- *Saussurea lappa*: The active constituents include palmitic acid, beta sitosterol, cynaropicrine, costunolide, alkaloid saussurine etc. the major uses of the plant are bitter, stomachic, diaphoretic, diuretic, disinfectant and is used to treat leprosy, hysteria, general debility, jaundice and cardiac disorders [76].
- *Rubia cordifolia*: The plant root is reported to be rich in resinous and extractive matter, gum, sugar, colouring matter. The principal constituent present is purpurine, glycoside manjistin, xanthine, garancin etc. the activities reported are; To treat Alzheimers disease, psychosis, leprosy, used for treating cardiac disorders etc [77].
- *Mesua ferrea*: Mesuol, mesuaxanthole-B, messuaferrol, sitosterol, mesuanic acid, α and β-amyrin, β sitosterol etc are the major constituents of the plant. The activities of the plant includes digestant, carminative, as a brain tonic, to treat hysteria, brain debility, scabies, skin disease, pruritis, leprosy, aphrodisiac, vomiting, dysentery and is an expectorant, vermicide and cardiac-tonic [78].
- *Punica granatum*: Used for treating heart diseases, Alzheimer's disease, anaemia, syphilis, diabetes, osteoarthritis, atherosclerosis and it is also used as an anti-inflammatory, anti-viral and anti-cancerous agent. The active constituents of the plant include; polyphenols like punicalagins, gallocatechins, polyphenol catechins, prodelphinidines, delphinidines and minerals like potassium [79].
- *Alpinia galanga*: The plant is used as an expectorant, anti-inflammatory, anti-arthritic, thermogenic, aromatic, stimulant, stomachic, nervine tonic, asthma, bronchitis, obesity, intermittent fever and diabetes [80].
- *Embelia ribes*: Embelin, Christembine, Homoembelin, Vilangine, Quercitol etc are the major constituents present in the plant. It is an effective astringent, stimulant, anti-infalmmatory, anti-bacterial agent and carminative. It is very effective in treating epilepsy and convulsions, ulcers, sore throat, insanity, cardiac failure, nervous debility, headache and tumor [81].

- *Abbies webbiana*: Methanol, Aziridine, flavanoids, glycosides and alkaloids are the principal constituents of the plant. It is used as an antibacterial, antimicrobial, antifungal agent and also cures cough, asthma, chronic bronchitis and allergic rhinitis [82].
- *Elettaria cardamomum*: the chief constituents of the plant includes protocatechu aldehyde, protocatechuic acid, alpha terpinyl acetate, limonene, linalool, cineole, terpenolene, myrcene, iron, calcium, copper, potassium etc. The uses of the plant include analgesic, anti-depressant and anti-cancerous activity [83].
- *Jasminum sambac*: Mixtures of coumarins, cardiac glycosides, essential oils, flavonoids, phenolics, saponins, and steroids are the major constituents of the plants. The medicinal properties of the plant includes; antiseptic, sedative, anti-depressant, diaphoretic, galactogogue, aphrodisiac, antispasmodic, expectorant, uterine etc. it is used for the treatment of eye disorders, jaundice, ulcers and skin diseases [84].
- *Nymphea stellata*: Apomorphine, phytosterols, nuciferin, phosphoesterase, biflavanoids, sucrose, fructose, glucose, mannitol, raffinose, galacturonic acid and amino acids are the chief constituents of the plant and the activities include euphoric, pain reliever, memory enhancer, coolant, bitter, used to treat jaundice, diarrhea, dysentery etc [85].
- *Baliospermum montanum*: Glycoterpenoids, steroids, flavonoids, titerpenoids, diterpenes, saponins and flavonoids are the chief chonstituents and the plant is used as an anti-inflammatory, anti-rheumatic, anti-dropsy, stimulant, purgative, rubefacient etc. It acts as a natural wound healer and natural detoxifier of the skin [86].

## 3. 8. Preparation of Formulation

The ingredients as described in Table-2 were coarsely powdered and one part of the powder was boiled in 16 parts of water. The mixture was then reduced to 4 parts.

## 3. 9. Method of storage

Store in airtight container in dry place.

## 3. 10. Ayurvedic properties

- Roga Karma: Unmada (schizophrenia), apasmara (epilepsy), jwara (fever), shukra kshaya (oligospermia), arthawa kshaya (reduced ovum production), medha kshaya (loss of memory and intelligence), pandu (discoloration of skin) and agnimandya (slow digestion).
- Dosha Karma (active against): Tridoshahara (destroys three doshas)
- Agni Karma (digestive function): Deepana (carminative)
- Reference: Ashtangahrudayam Uttara Tantra 6/26.
- Manufacturer: SNA Oushadhasala Pvt Ltd [87].

# 4. AIM AND OBJECTIVES

## 4. 1. AIM:

Kalyanakam Kashayam, an Ayurvedic concentrated decoction is prepared out of 27 different herbal ingredients, which consist of water soluble active principles. It is reported to be effective in the treatment of various neurological disorders, epilepsy, psychosis, anxiety etc. The literature study done so far revealed that there is a lack of scientific data concerning the neuropsychopharmacological evaluation of the formulation on different animal models.

The aim of the current study is to assess the neuropsychopharmacological effect of the Ayurvedic formulation in Swiss Albino mice.

## 4. 2. OBJECTIVES:

The objectives of the present study include:

- Evaluation of *in vitro* antioxidant activity of the formulation.
- Acute toxicity study of Kalyanakam kashayam.
- Neuropsychopharmacological evaluation of the Ayurvedic formulation in Swiss Albino mice.

# 5. PLAN OF WORK

The present study examines the efficacy of Kalyanakam kashayam an Ayurvedic formulation in CNS disorders. The effect of the drug was evaluated on various animal models, which was designed as follows.

- Literature search and Selection of the Formulation.
- Selection of suitable vehicle and dosage of the formulation.
- In vitro Antioxidant study.
- Acute toxicity study.
- Evaluation of Neuropsychopharmacological effect of Kalyanakam Kashayam using different animal models.

## ❖ DEPRESSION

a) Pentobarbitone induced sleeping time
b) Exploratory behavior-Actophotometer
c) Forced swim test

## ❖ ANXIETY

a) Elevated plus maze model
b) Hole board test

## ❖ NEUROTOXICITY

a) Rotarod test

## ❖ SCHIZOPHRENIA

a) Apomorphine induced stereotypy

## ❖ CATATONIA

a) Haloperidol induced catalepsy

## ❖ CONVULSION

a) Pentylenetetrazole - Induced Convulsions

- Statistical analysis.
- Documentation of results.

# 6. MATERIALS AND METHODS

## 6.1. Collection of the Formulation

Kalyanakam Kashayam (Batch number- F0876) was procured from SNA Oushadhasala Pvt. Ltd, Kerala.

## 6. 2. Experimental Animals

Swiss Albino mice (25-30g, either sex) were used for experiments. The animals were provided by the Animal House, KMCH College of Pharmacy, Coimbatore, Tamilnadu. All the animals were housed for acclimatization for a period of at least 7 days in the laboratory animal room prior to study. They were fed with water and standard laboratory food *ad libitum* and were maintained under standard conditions of temperature, humidity and light (12 hours light/ 12 hours dark cycle). The animals were housed in poly-propylene cages in a group of five per cage. It was made sure that all the observations were made in noiseless, diffusely illuminated room between 9.00 to 17.00 hr in the experimental room. All the experimental procedures were approved by the Institutional Animal Ethical Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), New Delhi, India.

## 6. 3. Requirements

**Table-3: Requirements of the study**

| DRUGS | CHEMICALS | OTHERS |
|---|---|---|
| 1. Apomorphine | 1.ABTS | 1.Distilled water |
| 2.Chlorpromazine | 2. DPPH | 2.Sterile water for injection |
| 3.Diazepam | 3.Phosphate Buffer | 3.Normal saline |
| 4.Haloperidol | 4.Quercetin | 4.Syringe |
| 5.Pentobarbitone Sodium | 5. Potassium Persulfate | 5.Needle |
| 6.Pentylenetetrazol | 6.FRAP Reagent | 6.Gloves |
| 7.Phenytoin | 7.Potassium Ferricyanide | |
| | 8.Ferric Chloride | |
| | 9.Gallic Acid | |
| | 10.Sodium Carbonate | |
| | 11.Folin Ciocalteau Reagent | |

# 7. EXPERIMENT DESIGN

- In-Vitro Anti-oxidant assay
- Acute Toxicity study
- Animal study

## 7. 1. In-Vitro Antioxidant Assay

### 7. 1. 1. DPPH radical scavenging activity [88] [89]

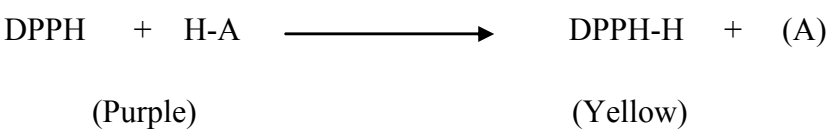
The free radical scavenging activity of the formulation was determined by DPPH method.

**REAGENTS:**

- 0.3mM DPPH (1, 1-diphenyl-2-picrylhydrazyl)
- Ethanol
- Quercetin(1mg/ml)
- Formulation(0.999mg/ml)

**PRINCIPLE:**

The reducing agent or the anti-oxidant reduces 1, 1- diphenyl picrylhydrazyl (purple chromogen radical DPPH•) to a hydrazine derivative (pale yellow). The absorbance of the mixture is measured at 519 nm and the decrease in absorbance of DPPH chromophore indicates a superior scavenging activity.



**PROCEDURE:**

DPPH radical scavenging assay was carried out spectrophotometrically. 1ml of 0.3mM ethanolic solution of DPPH was mixed with test solution dissolved in water at different concentrations like 9.99, 14.985, 19.98, 24.975, 49.95 and 99.9µg/ml.

The standard used for the assay was Quercetin (1mg/ml) and an equal volume of ethanol was taken as control. The reaction mixture was set aside in a dark room for 20 minutes. The drop in absorbance value (as a result of quenching of DPPH radical) was measured spectrophotometrically at 517nm. The experiment was done in duplicate and a parallel blank was also prepared excluding the sample/standard compound. The percentage inhibition was calculated as:

$$\text{Percentage inhibition (I \%)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} X\ 100$$

Where;

'$Abs_{control}$'= The absorbance of the control

'$A_{test}$' = The absorbance of sample/ standard.

$IC_{50}$ value of the sample was determined from percentage inhibition versus concentration curve. The lower $IC_{50}$ value indicates a better radical scavenging activity.

## 7. 1. 2. ABTS radical scavenging assay [90] [91]

**REAGENTS:**

- 7 mM ABTS salt [2, 2, - azinobis (3-ethylbenzoline-6-sulfonic acid)]
- 2.45mM Potassium per sulphate
- Phosphate buffer (pH 7.4)
- Quercetin (0.1mg/ml)
- Formulation (0.999mg/ml)

**PRINCIPLE:**

The assay assesses the ability of the sample/ standard compound to scavenge the long-lived cationic chromophore 2, 2'- azinobis- (3-ethylbenzothiazoline-6-sulphonate) ie ($ABTS^{\bullet+}$). The absorbance of the chromophore was measured at 734nm and the reduction in absorbance of chromophore indicates superior scavenging activity. The hydrogen atom donating ability of anti-oxidant helps them to scavenge $ABTS^{\bullet+}$ generated in aqueous phase and was measured spectrophotometrically.

**PROCEDURE:**

- **Preparation of phosphate buffer (pH. 7.4):**

0.2M Potassium Dihydrogen Phosphate dissolved in 50ml distilled water was mixed with 39.1ml Sodium Hydroxide solution (0.3128g NaOH in 39.1ml distilled water). The mixture was made upto a volume of 250ml with distilled water in a standard flask and the pH was adjusted to 7.4.

- **Preparation of ABTS radical cation:**

7mM stock solution of ABTS was mixed with 2.54mM potassium persulfate and was kept aside for 12-16 hours in a dark room at room temperature before use. ABTS solution was diluted with phosphate buffer (pH 7.4) to an absorbance of 0.7± (0.02) at 734nm.

The assay was carried out using different concentrations of the formulation like 9.99, 14.985, 19.98, 24.975, 49.95 and 99.9µg/ml and 1 ml ABTS solution was added to it. Absorbance of this mixture was read at 734nm after 6 minutes. Control was prepared excluding the sample. The standard used in the assay was quercetin at a concentration of 0.1 mg/ml. The experiment was done in duplicate and a parallel blank was also prepared excluding the sample/standard compound. The percentage inhibition was calculated as:

$$\text{Percentage inhibition (I \%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

Where;

'$Abs_{control}$'= The absorbance of the control

'$A_{test}$' = The absorbance of sample/ standard.

$IC_{50}$ value of the sample was determined from percentage inhibition versus concentration curve. The lower $IC_{50}$ value indicates a better radical scavenging activity.

## 7. 1. 3. Ferric Reducing Anti-oxidant Power Assay (FRAP assay) [92] [93]

**REAGENTS:**

- Acetate buffer (pH. 3.6)
- 20mMol TPTZ (2, 4, 6, - tripyridyl-5-triazine solution)
- 20mMol Ferric chloride
- Quercetin (1mg/ml)
- Formulation (0.999mg/ml)

**PRINCIPLE:**

The basic principle involved in the assay is the reduction of the ferric complex of $Fe(TPTZ)^{3+}$ i.e tripyridyl triazine (a ferroin analogue) to $Fe(TPTZ)^{2+}$ a ferrous complex (intensely blue in colour) in the presence of anti-oxidants in an acidic pH. There will be an increase in absorbance value at 593nm.

**PROCEDURE:**

**Preparation of FRAP reagent**

The reagent was prepared by mixing 25ml 0.2M acetate buffer (pH 3.6), 2.5ml of 20mMol TPTZ and 2.5 ml of 20mMol Ferric chloride.

The sample/standard compounds were taken in different concentrations and was mixed with 0.9ml of the reagent. Absorbance of the mixture was measured spectrophotometrically at 595nm after 30 minutes of incubation. Quercetin was used as standard. The experiment was done in duplicate and a parallel blank was also prepared excluding the sample/standard compound.

## 7. 1. 4. Reducing power assay [94] [95]

**REAGENTS:**

- Potassium Ferricyanide (1%)
- Ferric chloride (0.1%)
- 0.2M Phosphate buffer (pH. 6.6)
- Quercetin(1mg/ml)
- Formulation (0.999mg/ml)

**PRINCIPLE:**

The principle is based on the ability of the sample/standard to react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then forms ferric ferrous complex upon reaction with ferric chloride. The complex has an absorption maximum at 700 nm.

Potassium ferricyanide + Ferric chloride ⟶ Potassium ferrocyanide + ferrous chloride

**PROCEDURE:**

Determination of the reducing power of the extract/standard was done according to the method of Oyaizu (1986) with some modifications. The formulation at different concentrations 9.99, 14.985, 19.98, 24.975, 49.95 and 99.9µg/ml was made up to 250µl with distilled water and was treated with 250µl phosphate buffer (0.2M, pH. 6.6) and 250 µl potassium ferricyanide (1%). The mixture was then incubated for 20 minutes at 50˚C and 250 µl of 10% trichloro acetic acid was added to it. The tubes were centrifuged at 3000 rpm for 10 minutes and 500 µl of the supernatant was taken, mixed with an equal volume of distilled water and added with 100µl of 1% freshly prepared ferric chloride. The absorbance was measured at 700nm. The experiment was done in duplicate and a parallel blank was also prepared excluding the sample/standard compound. The changes in absorbance are proportional to the reductive ability.

## 7. 2. Total phenolic content [96]

The sample at a concentration of 9.99mg/ml was made upto 1000 µl with distilled water. To this mixture, 50 µl of Folin Ciocalteu and 860 µl of distilled water were added. The mixture was then incubated for 5 minutes and added 100 µl of sodium carbonate, to which 890µl of distilled water was added. The absorbance of the reaction mixture was measured at 725nm after 30 minutes of incubation. The experiment was done in duplicate. Gallic acid was used as the standard at a concentration of 100mg/ml and a calibration curve was plotted using the standard and sample values.

## 7. 3. Acute toxicity test

Acute oral toxicity study was performed as per OECD-423 guidelines. The animals were fasted overnight with free excess of water and were grouped into three consisting of 3 animals each, to which the formulation was administered orally at the dose level of 500mg/kg, 2000mg/kg and 5000mg/kg body weight. They were observed for mortality; toxic symptoms such as behavioral changes, locomotor activity, convulsions; direct observation parameters such as tremor, convulsion, salivation, diarrhoea, sleep, coma, changes in skin and

fur, eyes and mucous membrane, respiratory, circulatory, autonomic and CNS, somatomotor activity etc.

## 7. 4. ANIMAL STUDY

### 7. 4. 1. Animal models of Depression

### 7. 4. 1. A. Spontaneous Locomotor Activity using Actophotometer [97]

The spontaneous locomotor activity was measured using an actophotometer, which operates on photoelectric cells connected in circuit with a counter. A count was recorded and displayed, when a beam of light falling on the photocell was interrupted by the movement of the animal. Animals were randomly divided into three groups of 5 animals each (either sex). The mouse was placed individually inside the actophotometer chamber for noting the basal activity score for a period of 10 minutes. The formulation at doses of (250 and 500mg/kg, p. o) and the standard drug mephentramine (30mg/kg, i.p.) was given 30 minutes prior to the test after which the mice were placed again inside the chamber for measuring the activity.

**Experimental design:**

Group-1: Standard, received Mephentramine (30mg/kg; i.p.)

Group-2: Test, received Formulation (250mg/kg; p.o. low dose)

Group-3: Test, received Formulation (500mg/kg; p.o. high dose)

Percentage decrease in activities were calculated for each group using the formula,

$$\text{Percentage decrease in activity} = (1 - W_a / W_b) \times 100$$

Where, $W_a$ and $W_b$ are the average activity score before and after drug administration respectively and calculated.

## 7. 4. 1. B. Pentobarbitone Induced Sleeping Time [98]

The test was carried out in four groups of five mice each to which pentobarbitone sodium (40mg/kg) was administered intraperitonially, 30 minutes after the administration of standard and sample. The animals were observed for loss of righting reflex (inability to return to the upright position on all four limbs after the animal was placed on its back). The duration of sleep was measured by noting the time interval between the loss and recovery of righting reflex.

**Experimental design:**

Group-1: Control, received Pentobarbitone Sodium (40mg/kg; i.p.)

Group-2: Standard, received Chlorpromazine (3mg/kg; i.p.) + PB

Group-3: Test, received Formulation (250mg/kg; p.o. low dose) + PB

Group-4: Test, received Formulation (500mg/kg; p.o. high dose) + PB

## 7. 4. 1. C. Forced Swim Test [99]

The procedures for Forced swim test or Despair swim test were similar to those first described by Porsolt., *et al*. (1977). This test is sensitive to all major classes of antidepressant drugs, hence used as an animal model of depression like behaviour. Mice were grouped into three of five animals each and were trained individually for three consecutive days (pre-test session). In the "test-session" The animals were made to swim individually in an open cylindrical container of diameter 20cm and height 50cm, containing 25cm of water at 25˚C, for a test period of 6 minutes. After a brief period of vigorous activity for about two minutes, mice maintain a typical immobile posture. The animals were considered immobile when they float in an upright position, making only negligible movements to maintain their head above water. The total duration of immobility was recorded and changes in the same were studied for various treatment groups.

**Experimental design:**

Group-1: Standard, received Mephentramine (30mg/kg; i.p.)

Group-2: Test, received Formulation (250mg/kg; p.o. low dose)

Group-3: Test, received Formulation (500mg/kg; p.o. high dose)

## 7. 4. 2. Animal models of Anxiety

## 7. 4. 2. A. Elevated Plus Maze Model of Anxiety [100]

The plus maze for mice (Lister, 1987) consisted of two perpendicular open arms (30×5cm) and two closed arms (30×5×15cm) also in perpendicular position. The open and closed arms were connected with each other by a central platform (5×5cm). The lateral walls of the closed arm and floor of each arm were made of wood. The platform and lateral walls were painted in black. The maze was elevated at a height of 45cm above the floor. The animals were trained for two consecutive days (pre-test session). In the test-session, 30 minutes after the treatment with standard and sample, the animal was placed at the center of the maze with its nose facing in the direction of one of the closed arms. The duration of the test period was 10 minutes, during which the following parameters were evaluated.

- The number of entries into the open arm.
- The number of entries into the closed arm.
- Time spent in the open arm.
- Time spent in the closed arm.

The experiment was carried out in a sound attenuated room. An entry was counted when the animal placed all its four paws on an arm.

**Experimental design:**

Group-1: Control, received Normal saline

Group-2: Standard, received Diazepam (5mg/kg; i.p.)

Group-3: Test, received Formulation (250mg/kg; p.o. low dose)

Group-4: Test, received Formulation (500mg/kg; p.o. high dose)

## 7. 4. 2. B. Hole Board Apparatus [101]

Hole board apparatus is a wooden board of the size 40×40cm. The board consists of sixteen holes with a diameter of 3cm which are distributed evenly on the board. The board is elevated to a particular height (5 cm) from the floor, so that the mouse while poking its nose into the hole does not see the bottom. The mice were grouped into four of five animals each and were trained for two consecutive days. Thirty minutes after the administration of test and standard drugs, the animals were subjected to the test for a period of six minutes and the number of counts for nose-poking of treated animals was calculated as percentage of control animals.

**Experimental design:**

Group-1: Control, received Normal saline

Group-2: Standard, received Diazepam (5mg/kg; i.p.)

Group-3: Test, received Formulation (250mg/kg; p.o. low dose)

Group-4: Test, received Formulation (500mg/kg; p.o. high dose)

## 7. 4. 3. Animal model for Neurotoxicity

## 7. 4. 3. A. Rota rod Test [102]

Rota rod test was used to evaluate the effect of drug on motor coordination in mice. The instrument consists of a horizontal rotating rod (3cm diameter) rotating at a rate of 25rpm. During the pre-test session, the animals were kept on the rotating rod and those animals that had demonstrated their ability to remain on the rotating rod for at least three minute were used. In the test session, the experiment was repeated 30 minutes after the administration of formulation and standard drugs for a period of 5 minutes the fall off time for each animal was recorded. If the animal fails more than once to remain on the rotating rod for 5 minutes was considered to be neurotoxic.

**Experimental design:**

Group-1: Control, received Normal saline

Group-2: Standard, received Diazepam (5mg/kg; i.p.)

Group-3: Test, received Formulation (250mg/kg; p.o. low dose)

Group-4: Test, received Formulation (500mg/kg; p.o. high dose)

## 7. 4. 4. Apomorphine (APM) induced stereotypy in mice [103]

Apomorphine induces a stereotyped behaviour in rodents, characterized by licking, sniffing, and gnawing in a repetitive compulsive manner, which is an indication of striatal dopaminergic stimulation (Anden *et al*. 1967; Ernst 1967; Costall and Naylor 1973). The animals were grouped into three of five animals each and were trained for three consecutive days. In the test groups, mice were treated with the formulation 30 minutes prior to apomorphine administration. The test group and control group animals were placed in plastic or glass cage

individually and the intensity of stereotyped behavior was assessed at 10 minutes interval for 50 minutes. Scoring was done as follows:

Score 0: no changes than control.

Score 1: discontinuous sniffing, constant exploratory activity.

Score 2: continuous sniffing, periodic exploratory activity.

Score 3: continuous sniffing, discontinuous biting, gnawing or licking, very brief periods of locomotor activity.

Score 4: continuous biting, gnawing or licking, no exploratory activity.

**Experimental design:**

Group-1: Control, received Apomorphine (1mg/kg; i.p.)

Group-2: Test, received APM + Formulation (250mg/kg; p.o. low dose)

Group-3: Test, received APM + Formulation (500mg/kg; p.o. high dose)

## 7. 4. 5. Haloperidol (HP) – induced catalepsy in mice [104]

Catalepsy may be defined as a nervous condition characterized by muscular rigidity and fixity of posture regardless of external stimuli, as well as a decreased sensitivity to pain. Haloperidol (1mg/kg; i.p.) was used to induce catalepsy. The dose was selected as 1mg/kg so that the drug could elicit a moderate degree of catalepsy thereby making the detection of either termination or potentiation of the phenomenon.

The animals were grouped and numbered and were trained for three successive days. In the test session, animals were treated with formulation 30 minutes prior to haloperidol administration and the forelegs of the mice were placed over a horizontal bar elevated at a height of 3cm and 5cm respectively after 30 minutes of haloperidol injection. The cataleptic behaviour was scored as; point 1 for animals which maintained the imposed posture

for at least 20 seconds. For every further 20 seconds the animal maintained the cataleptic posture, was awarded with extra point and so on. The endpoint of catalepsy was considered to occur when the animal removed both front paws from the horizontal bar or if the animal moved its head in an exploratory manner.

**Experimental design:**

Group-1: Control, received Haloperidol (1mg/kg; i.p.)

Group-2: Test, received HP + Formulation (250mg/kg; p.o. low dose)

Group-3: Test, received HP + Formulation (500mg/kg; p.o. high dose)

## 7. 4. 6. Pentylenetetrazol (PTZ) – induced convulsions in mice [105]

The mice were divided into 4 groups of five animals each. The animals were injected with Pentylenetetrazol (80mg/kg) 30 minutes after formulation and standard drug (Diazepam 4mg/kg) administration. Each animal was placed in separate plastic cage for observation. The occurrence of the first generalized clonus (repeated clonic seizures of the fore and hind limbs lasting over 5-10 seconds) or jerky movements were recorded during individual observation for 30 minutes. The onset and duration both were observed. Reduction in such movements was selected as criteria for supporting antiepileptic activity of the drug.

**Experimental design:**

Group-1: Control, received PTZ (80mg/kg; i.p.)

Group-2: Standard, received Diazepam (4mg/kg; i.p.) + PTZ.

Group-3: Test, received Formulation (250mg/kg; p.o. low dose) + PTZ.

Group-4: Test, received Formulation (500mg/kg; p.o. high dose) + PTZ.

## 7. 5. Statistical Analysis

All the data were expressed as the mean ± standard error of the mean (SEM). The statistical significance of the differences between the groups was analyzed by using GraphPad 5.0 software (GraphPad, San Diego, USA) by applying one way Analysis Of Variance (ANOVA) followed by Dunnett's test as post hoc and also Student's Paired T-test. The values of $P<0.05$ was considered to be statistically significant.

# 8. RESULTS

## 8. 1. In-Vitro Antioxidant Assay

### 8. 1. 1. DPPH radical scavenging activity

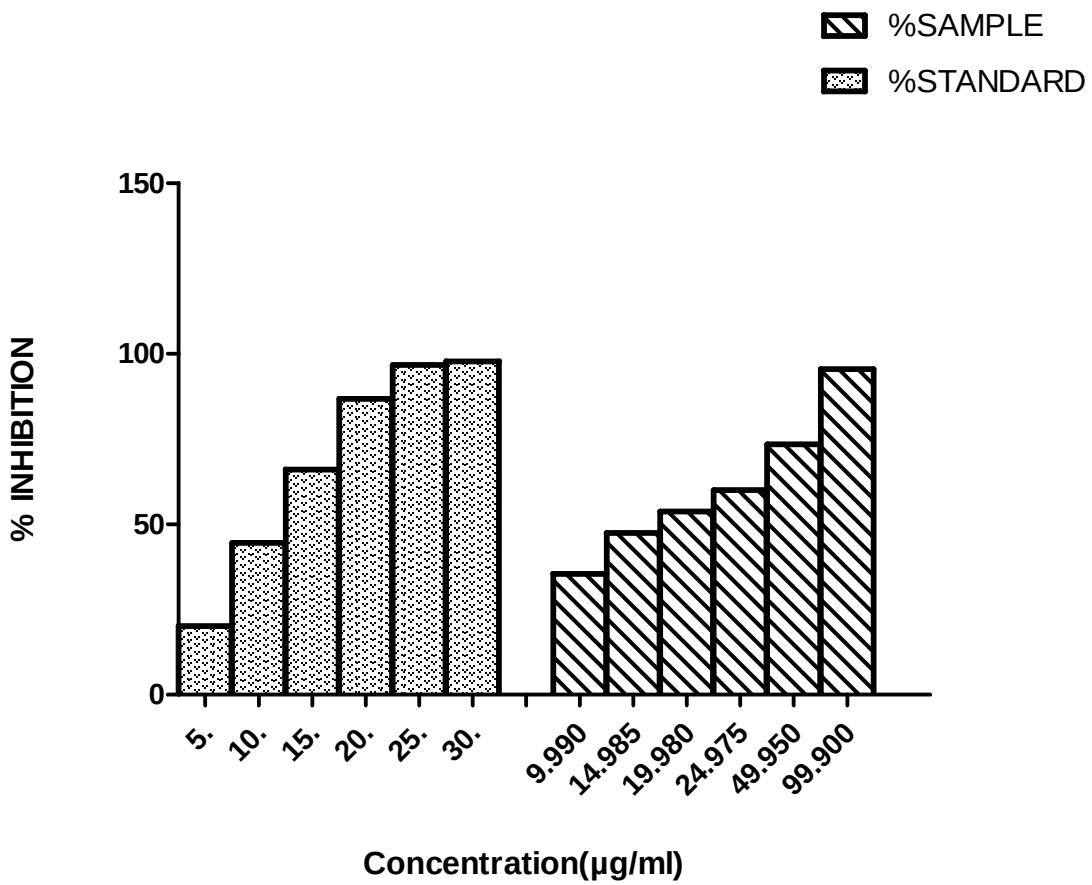
DPPH radical scavenging assay was performed using different concentrations of sample and standard (Quercetin), and the results are shown in table: 4 and figure: 1.

**Table-4: Comparitive DPPH radical scavenging activity of Quercetin and formulation.**

| TREATMENT | CONCENTRATION (µg/ml) | ABSORBANCE | PERCENTAGE INHIBITION (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| **STANDARD (QUERCETIN)** | 5 | 1.93 ± 0.021 | 20.23 | |
| | 10 | 1.003 ± 0.029 | 44.50 | |
| | 15 | 0.8626 ± 0.041 | 66.08 | 10.38 µg/ml |
| | 20 | 0.5788 ± 0.007 | 86.82 | |
| | 25 | 0.2895 ± 0.011 | 96.69 | |
| | 30 | 0.2522 ± 0.001 | 97.84 | |
| **SAMPLE** | 9.990 | 1.307 ± 0.015 | 35.520 | |
| | 14.985 | 1.170 ± 0.008 | 47.390 | |
| | 19.980 | 0.908 ± 0.021 | 53.780 | 17.14µg/ml |
| | 24.975 | 0.798 ± 0.007 | 60.010 | |
| | 49.950 | 0.551 ± 0.014 | 73.430 | |
| | 99.900 | 0.153 ± 0.006 | 95.450 | |

Values are expressed as mean ± S.E.M; (n = 2).

**Figure-1: Radical scavenging activity of Quercetin and formulation at various concentrations**




The results of the radical scavenging assay are expressed in percentage (%) inhibition of DPPH free radical.

## 8. 1. 2.ABTS radical scavenging assay

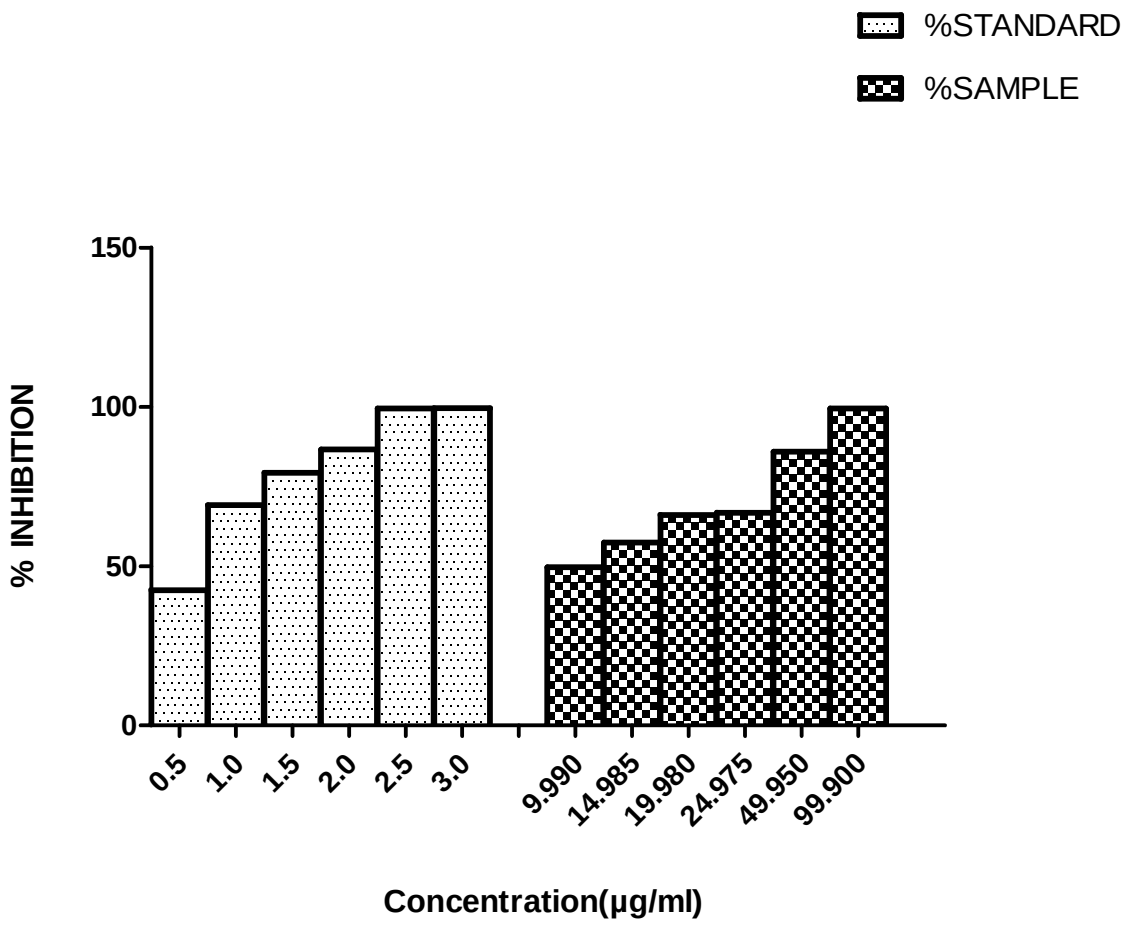
ABTS radical scavenging assay was performed using different concentrations of sample and standard (Quercetin), and the results are shown in table: 5 and figure: 2.

**Table-5: Comparitive ABTS radical scavenging activity of Quercetin and formulation.**

| TREATMENT | CONCENTRATION (µg/ml) | ABSORBANCE | PERCENTAGE INHIBITION (%) | $IC_{50}$ (µg/ml) |
|---|---|---|---|---|
| **STANDARD (QUERCETIN)** | 0.5 | 0.2630 ± 0.006 | 42.50 | |
| | 1.0 | 0.2169 ± 0.013 | 69.15 | |
| | 1.5 | 0.1447 ± 0.008 | 79.37 | 0.6149µg/ml |
| | 2.0 | 0.0977 ± 0.011 | 86.64 | |
| | 2.5 | 0.0037 ± 0.0035 | 99.47 | |
| | 3.0 | 0.003 ± 0.004 | 99.57 | |
| **SAMPLE** | 9.990 | 0.325 ± 0.014 | 49.68 | |
| | 14.985 | 0.299 ± 0.11 | 57.38 | |
| | 19.980 | 0.238 ± 0.009 | 66.08 | 11.29µg/ml |
| | 24.975 | 0.233 ± 0.007 | 66.79 | |
| | 49.950 | 0.099 ± 0.015 | 85.89 | |
| | 99.900 | 0.004 ± 0.003 | 99.43 | |

Values are expressed as mean ± S.E.M; (n = 2).

**Figure-2: Radical scavenging activity of Quercetin and formulation at various concentrations**




The results of the radical scavenging assay expressed in percentage (%) inhibition of ABTS free radical.

## 8. 1. 3.Ferric Reducing Anti-oxidant Power Assay (FRAP assay)

Ferric reducing anti-oxidant powerassay was performed using different concentrations of sample and standard (Quercetin), and the results are shown in table: 6.

**Table-6: Comparitive Ferric reducing ability of standard and sample**

| TREATMENT | CONCENTRATION (µg/ml) | ABSORBANCE | REDUCING POWER (%) |
|---|---|---|---|
| **STANDARD (QUERCETIN)** | 5 | 0.3660 ± 0.0183 | 51.87 |
| | 10 | 0.5748 ± 0.008 | 64.20 |
| | 15 | 0.7873 ± 0.007 | 70.05 |
| | 20 | 1.0687 ± 0.011 | 81.64 |
| | 25 | 1.2997 ± 0.005 | 93.18 |
| | 30 | 1.5494 ± 0.013 | 98.75 |
| **SAMPLE** | 9.990 | 0.2523 ± 0.021 | 43.35 |
| | 14.985 | 0.2692 ± 0.007 | 52.95 |
| | 19.980 | 0.2865 ± 0.003 | 62.75 |
| | 24.975 | 0.3012 ± 0.008 | 71.08 |
| | 49.950 | 0.3201 ±0.018 | 81.875 |
| | 99.900 | 0.3403 ± 0.005 | 93.35 |

Values are expressed as mean ± S.E.M; (n = 2).

## 8. 1. 4.Reducing power assay

Reducing power assaywas performed using different concentrations of sample and standard (Quercetin), and the results are shown in table: 7.

**Table-7: Comparitive reductive ability of standard and sample**

| TREATMENT | CONCENTRATION (µg/ml) | ABSORBANCE | REDUCING POWER (%) |
|---|---|---|---|
| **STANDARD (QUERCETIN)** | 5 | 0.3357± 0.008 | 48.73 |
| | 10 | 0.3503 ± 0.013 | 55.20 |
| | 15 | 0.3741 ± 0.003 | 65.75 |
| | 20 | 0.4007 ± 0.007 | 77.53 |
| | 25 | 0.4207 ± 0.015 | 86.39 |
| | 30 | 0.4399 ± 0.008 | 94.90 |
| **SAMPLE** | 9.990 | 0.2995 ± 0.019 | 47.520 |
| | 14.985 | 0.4104 ± 0.003 | 54.400 |
| | 19.980 | 0.3485 ± 0.021 | 63.620 |
| | 24.975 | 0.3693 ± 0.034 | 75.050 |
| | 49.950 | 0.3951 ± 0.004 | 81.560 |
| | 99.900 | 0.4098 ± 0.003 | 96.320 |

Values are expressed as mean ± S.E.M; (n = 2).

## 8. 2.Total phenolic content

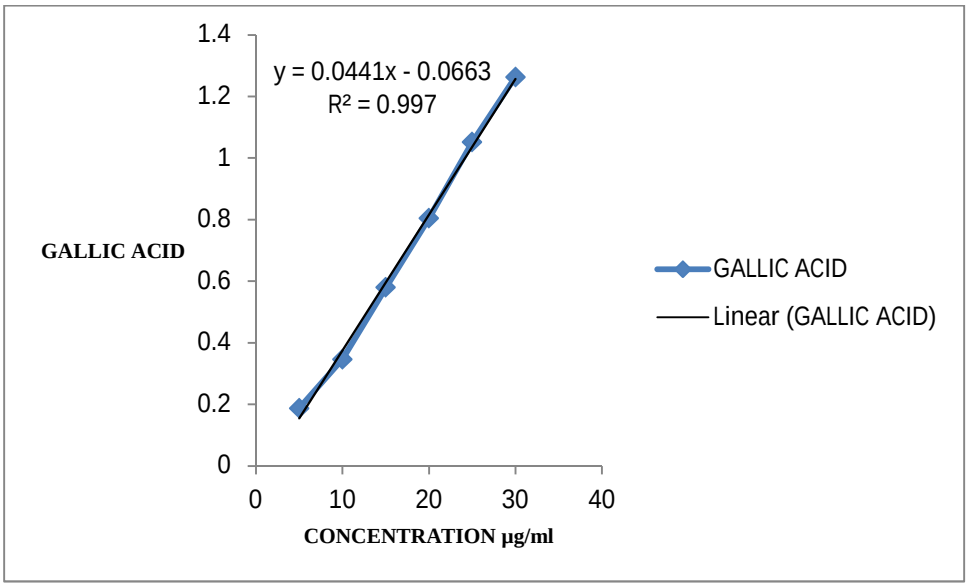
Phenolic compounds may contribute directly to the anti oxidative action. The total phenolic content of the formulation was quantified using different concentrations of the standard (Gallic acid) and the sample. The results are summarized in table-8 and figure-3

.

**Table-8: Determination of Total phenolic content of the formulation**

| TREATMENT | CONCENTRATION (µg/ml) | ABSORBANCE |
|---|---|---|
| **STANDARD (GALLIC ACID)** | 5 | 0.1875 |
| | 10 | 0.3463 |
| | 15 | 0.5799 |
| | 20 | 0.8049 |
| | 25 | 1.0521 |
| | 30 | 1.2629 |
| **SAMPLE** | 9990 | 1.8811 |

**Figure-3: Total phenolic content of the sample**




The total phenolic content of the sample was found to be 44.29mg/g calculated as Gallic acid equivalent.

## 8. 3. Acute toxicity test

The acute toxicity test was performed by using the kashyam at concentrations 500mg/kg, 2000mg/kg and 5000mg/kg. The observations are summarized in table: 9.

## Table- 9: Acute toxicity study

| Sl. No. | Responses | FORMULATION | | | | | |
|---|---|---|---|---|---|---|---|
| | | Head | | Body | | Tail | |
| | | Before | After | Before | After | Before | After |
| 1 | Alertness | Normal | Normal | Normal | Normal | Normal | Normal |
| 2 | Grooming | Absent | Absent | Absent | Absent | Absent | Absent |
| 3 | Touch response | Absent | Absent | Absent | Absent | Absent | Absent |
| 4 | Torch response | Normal | Normal | Normal | Normal | Normal | Normal |
| 5 | Pain response | Normal | Normal | Normal | Normal | Normal | Normal |
| 6 | Tremor | Absent | Absent | Absent | Absent | Absent | Absent |
| 7 | Convulsion | Absent | Absent | Absent | Absent | Absent | Absent |
| 8 | Righting reflux | Normal | Normal | Normal | Normal | Normal | Normal |
| 9 | Gripping strength | Normal | Normal | Normal | Normal | Normal | Normal |
| 10 | Pinna reflux | Present | Present | Present | Present | Present | Present |
| 11 | Corneal reflux | Present | Present | Present | Present | Present | Present |
| 12 | Writhing | Absent | Absent | Absent | Absent | Absent | Absent |
| 13 | Pupils | Normal | Normal | Normal | Normal | Normal | Normal |
| 14 | Urination | Normal | Normal | Normal | Normal | Normal | Normal |
| 15 | Salivation | Normal | Normal | Normal | Normal | Normal | Normal |
| 16 | Skin colour | Normal | Normal | Normal | Normal | Normal | Normal |

## 8. 4. Effect of the Formulation on Spontaneous Locomotor Activity using Actophotometer in mice.

The locomotor activity of the animal was assessed using the standard (Mephentramine) and formulation. The datas are shown in table-10 and figure -4.

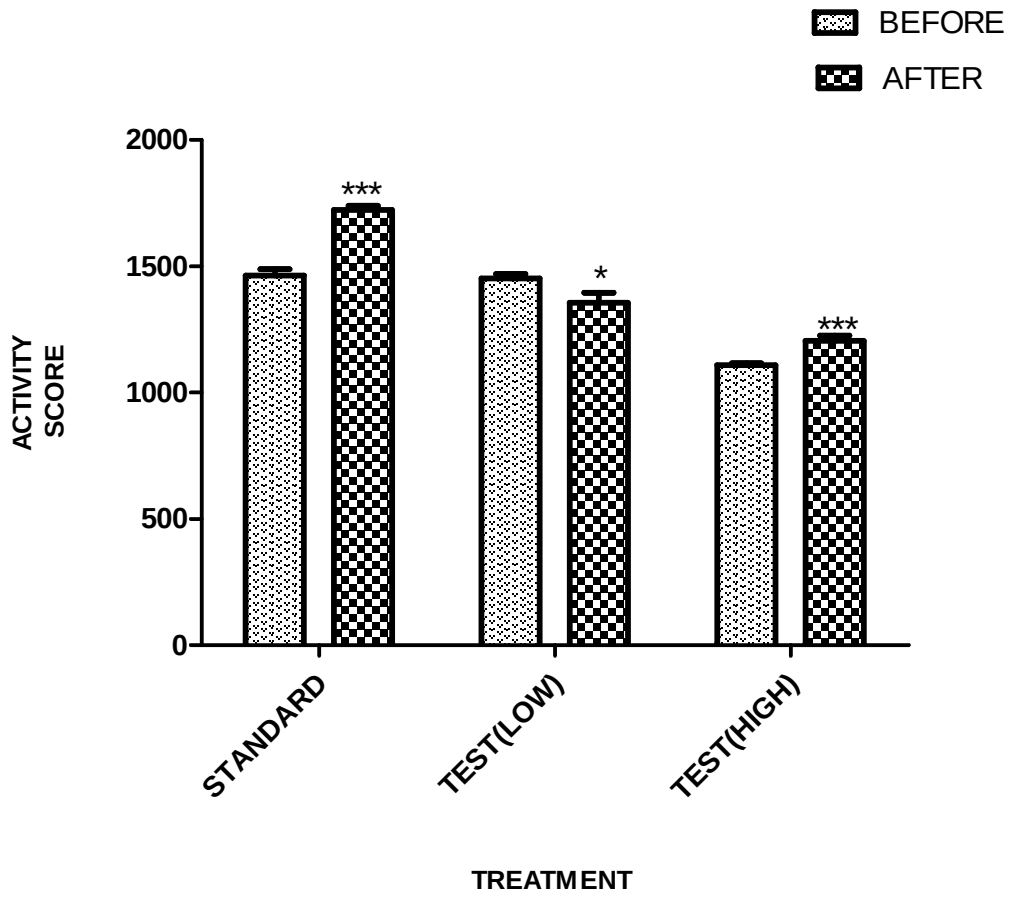
**Table – 10**:**Effect of the Formulation on Spontaneous Locomotor Activity**

| TREATMENT | PHOTOCELL ACTIVITY COUNT | |
|---|---|---|
| | BEFORE | AFTER |
| STANDARD (Mephentramine 30mg/kg) | 1463 ± 25.90 | 1723 ± 15.83*** |
| TEST(LOW) (250mg/ml) | 1452 ± 17.32 | 1355 ± 40.70* |
| TEST(HIGH) (500mg/ml) | 1109 ± 7.633 | 1205 ± 21.26*** |

Values were expressed as mean ± SEM. Statistical analysis was done by student's t-test (Paired t-test).

***P<0.001; *P<0.05; N = 5.

**Figure-4: Effect of the Formulation on Spontaneous Locomotor Activity in mice.**




Effect of the Formulation and standard on Spontaneous Locomotor Activity.Values were expressed as mean ± SEM. ***P<0.001; *P<0.05; N = 5.

## 8. 5. Effect of Kayanakam kashayam on Pentobarbitone – induced sleeping time in mice.

The formulation in the given doses (250mg/kg and 500mg/kg) showed significant potentiation of sleeping time induced by Pentobarbitone. The sleeping time of mice treated with Pentobarbitone alone and in combination with the standard andformulationare shown in table-11 and figure-5.

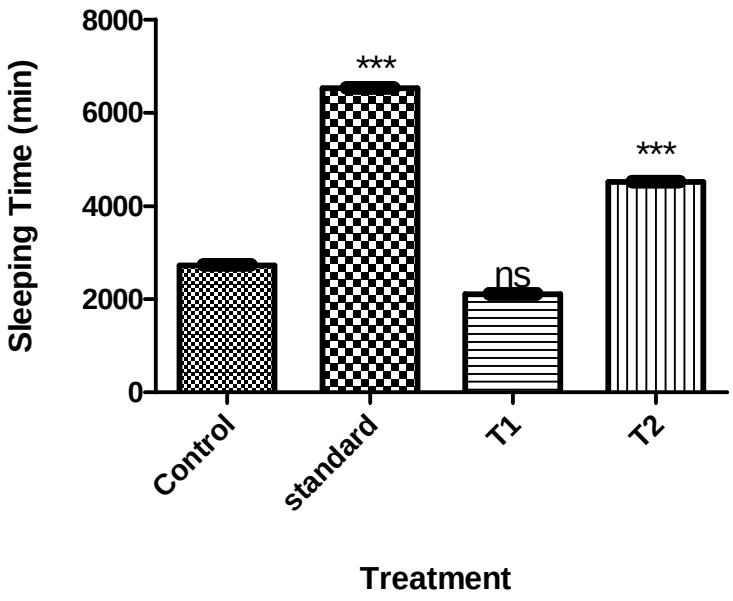
**Table – 11: Effect of the formulation on Pentobarbitone – induced sleeping**

| TREATMENT | SLEEPING TIME(seconds) |
|---|---|
| CONTROL | 2722 ± 15.62 |
| STANDARD (chlorpromazine 3mg/kg) | 6526 ± 7.48*** |
| TEST (Low dose-250mg/ml) | 2108 ± 7.35ns |
| TEST (High dose-500mg/ml) | 4514 ± 6.00*** |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

**Figure-5: Effect of the formulation on Pentobarbitone – induced sleeping time in mice.**




Effect of the formulation on Pentobarbitone – induced sleeping.Values were expressed as mean ± SEM. ***P<0.001; ns= non-significant; N = 5

Where, T1 = Formulation low dose (250 mg/kg)

T2 = Formulation high dose (500 mg/kg)

## 8. 6. Effect of the Formulation on Forced swim test in mice

The activity of the formulation was assessed at doses 250 and 500 mg/kg and the data are shown in table-12 and figure -6.

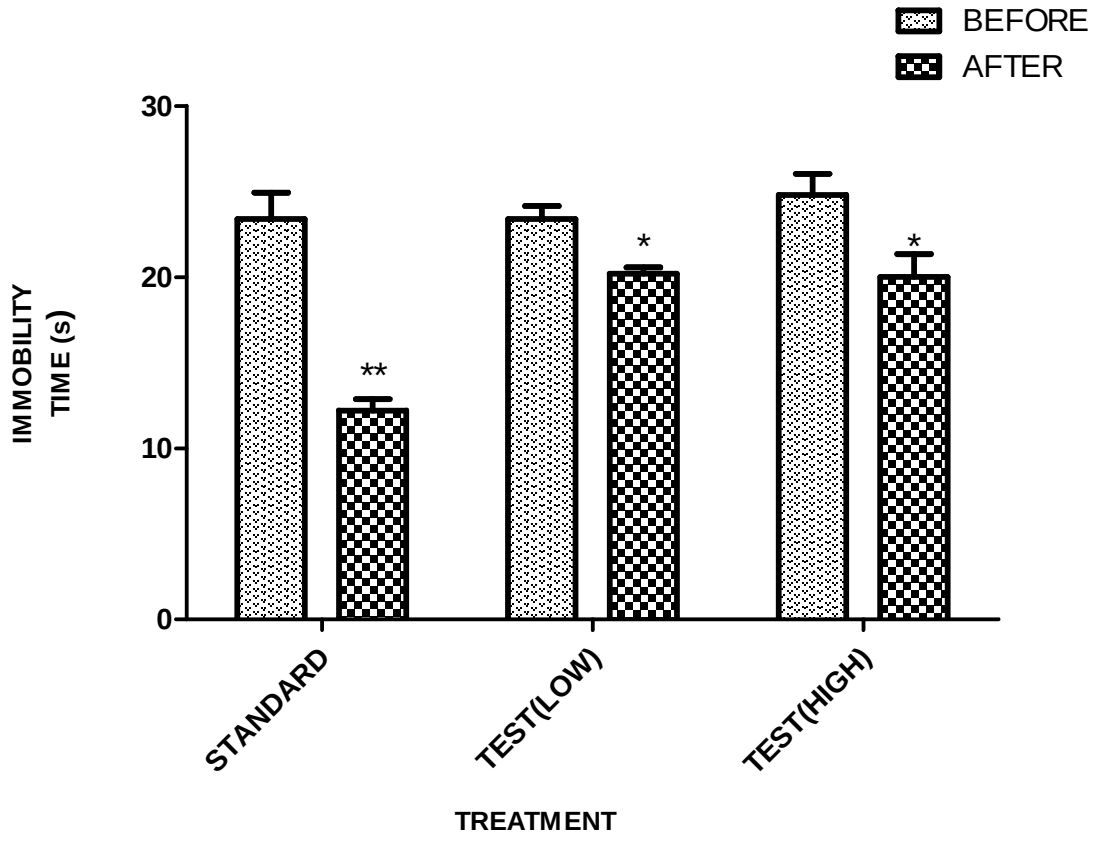
**Table – 12: Effect of the Formulation on Forced swim test**

| TREATMENT | DURATION OF IMMOBILITY(SECONDS) | |
|---|---|---|
| | BEFORE | AFTER |
| MEPHENTRAMINE(30mg/kg) | 23.40 ± 1.54 | 12.20 ± 0.66** |
| TEST(Low dose-250mg/ml) | 23.40 ± 0.75 | 20.20 ± 0.37* |
| TEST(High dose-500mg/ml) | 24.80 ± 1.24 | 20.00 ± 1.34* |

Values were expressed as mean ± SEM. Statistical analysis was done by Student's paired t-test.

**P<0.01; *P<0.05; N = 5.

**Figure-6: Effect of the Formulation on Forced swim test in mice**




Effect of the Formulation on Forced swim test.Values were expressed as mean ± SEM. **P<0.01; *P<0.05; N = 5.

## 8. 7. Effect of the Formulation on Elevated Plus Maze model of anxiety in mice

The anti-anxiety effect of the formulation was evaluated at doses 250 and 500 mg/kg.The data are shown in table-13& 14.

- **Before treatment**

**Table – 13: Elevated plus maze model of anxietybeforetreatment with formulation**

| TREATMENT | MEAN NO OF ENTRIES IN | | MEAN TIME SPENT IN | |
|---|---|---|---|---|
| | OPEN ARM | CLOSED ARM | OPEN ARM | CLOSED ARM |
| CONTROL | 9.60± 1.44 | 20.20 ± 3.43 | 59.40 ± 11.07ns | 425.8 ± 34.16ns |
| STANDARD | 9.00 ± 1.92ns | 23.60 ± 3.89ns | 112.8 ± 30.79ns | 396.4 ±48.38ns |
| TEST (Low dose-250mg/ml) | 9.8 ± 1.11ns | 21.00 ± 2.51ns | 40.00 ± 27.39ns | 514.0 ± 33.54ns |
| TEST(High dose-500mg/ml) | 9.4 ± 2.69ns | 21.80 ± 4.35ns | 39.60 ± 18.14ns | 509.6 ± 29.89ns |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.ns = non significant; N = 5

- **After treatment**

**Table – 14: Elevated plus maze model of anxiety after treatment with formulation**

| TREATMENT | MEAN NO OF ENTRIES IN | | MEAN TIME SPENT IN | |
|---|---|---|---|---|
| | OPEN ARM | CLOSED ARM | OPEN ARM | CLOSED ARM |
| CONTROL | 8.0 ± 1.58 | 10.80 ±2.39 | 100.6±7.31 | 429.8±31.92 |
| STANDARD (Diazepam 5mg/kg) | 10.00 ± 2.92ns | 13.60 ± 3.43ns | 260.6±12.78 *** | 331.2±14.47ns |
| TEST(Low dose-250mg/ml) | 15.0 ± 1.67ns | 14.40 ± 2.40ns | 205 ± 20.45** | 305.8±33.38ns |
| TEST(High dose-500mg/ml) | 10.20 ± 1.24ns | 12.80 ± 1.49ns | 220.2±24. 98 *** | 351.6±13.89ns |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; **P<0.01; ns= non significant; N = 5.

## 8. 8. Effect of Kalyanakam kashayam on Hole board test apparatus in mice

The number of nose poking made by the animal after the administration of standard and formulationwere compared with the control group for evaluating the anti-anxiety effect of formulation. The data obtained are shown in table-15 and figure -7.

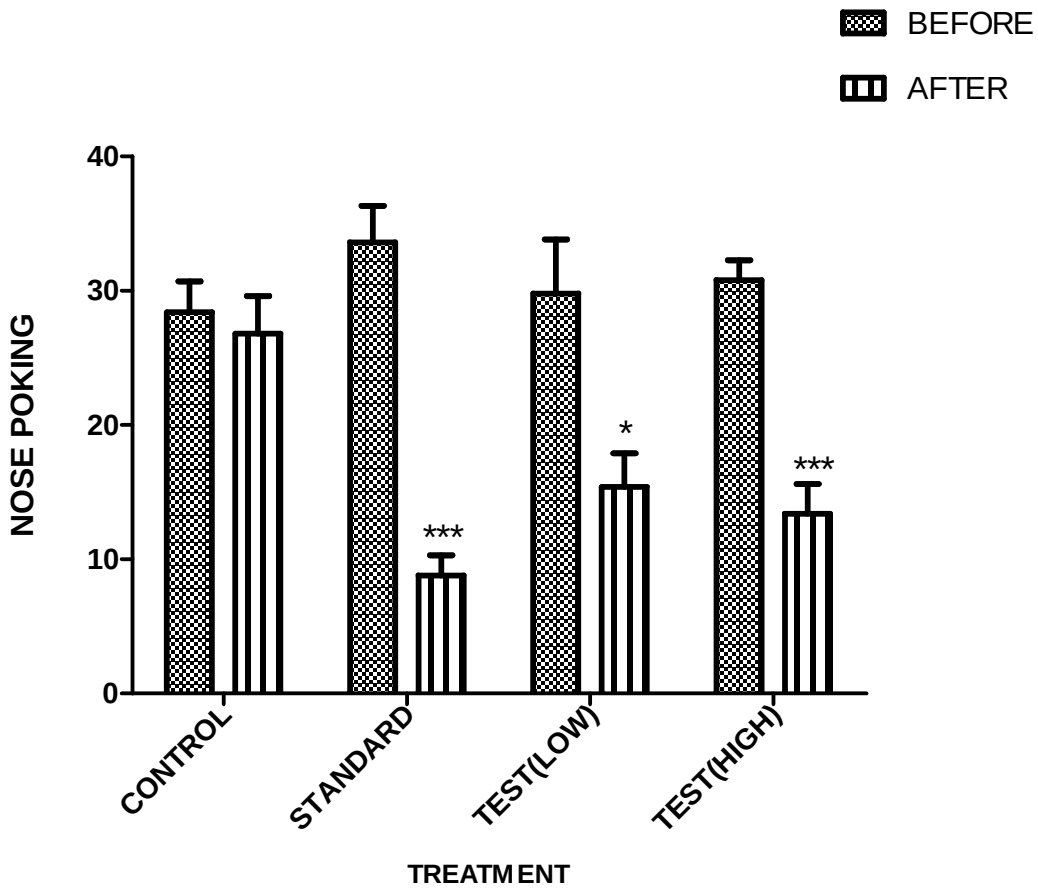
**Table – 15: Effect of the sample on nose poking behavior in mice**

| **TREATMENT** | **NUMBER OF NOSE POKING** | |
|---|---|---|
| | BEFORE | AFTER |
| CONTROL | 28.40 ± 2.293 | 26.80 ± 2.800 |
| DIAZEPAM(5mg/kg) | 33.60 ± 2.731 | 8.800 ± 1.497*** |
| TEST(Low dose-250mg/ml) | 29.80 ± 4.017 | 15.40 ± 2.482* |
| TEST(High dose-500mg/ml) | 30.80 ± 1.463 | 13.40 ± 2.205*** |

Values were expressed as mean ± SEM. Statistical analysis was done by Student's paired t-test.

***P<0.001; *P<0.05; N = 5.

**Figure-7: Effect of the Formulation on Hole board test apparatus in mice**




Values were expressed as mean ± SEM. ***P<0.001; *P<0.05; N = 5.

## 8. 9. Effect of the Formulation on Motor- coordination test using Rotarod apparatus in mice.

The effect of the formulation at doses 250 and 500 mg/kg on motor coordination was compared with the control group and the standard group (Diazepam5 mg/kg). The data obtained are shown in table-16 and figure-8.

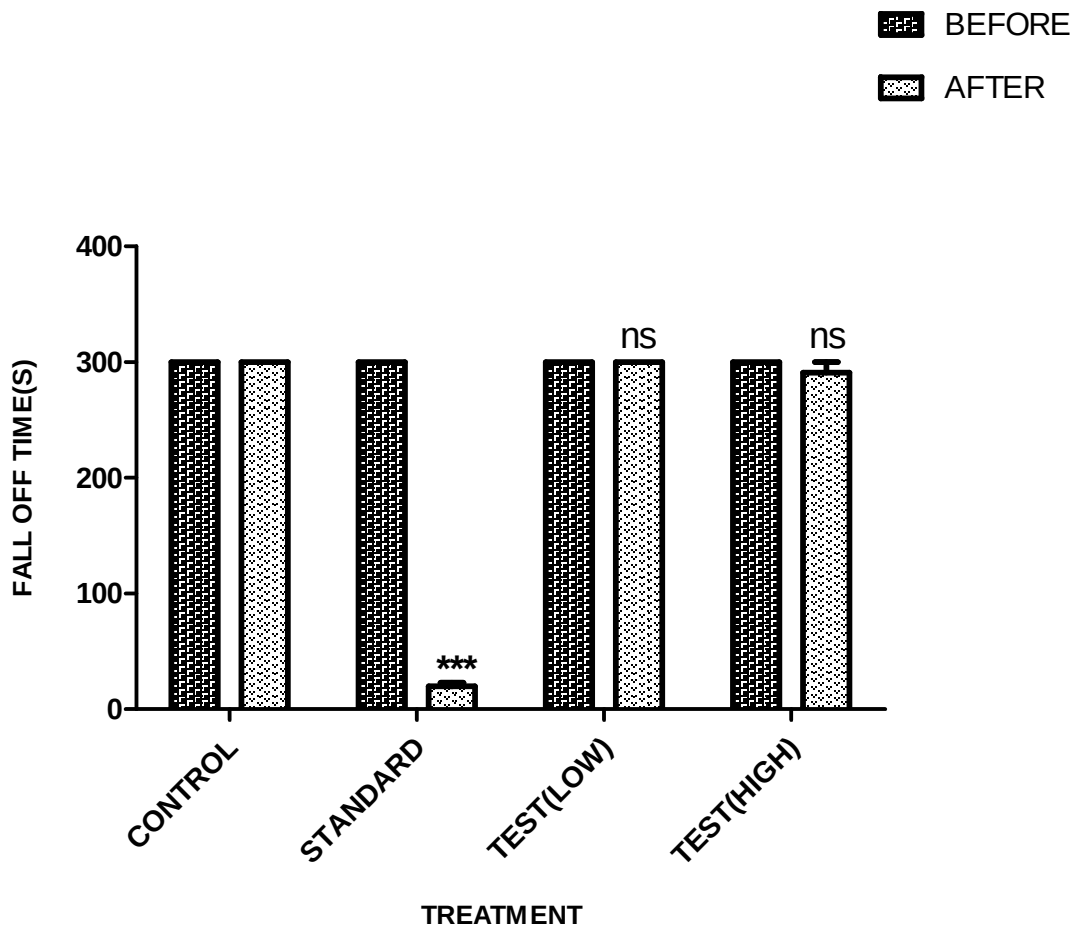
**Table-16: Effect of the Formulation on Motor- coordination test**

| TREATMENT | FALL OFF TIME(S) | |
|---|---|---|
| | BEFORE | AFTER |
| CONTROL | 300 | 300 |
| DIAZEPAM(5mg/kg) | 300 | 19.80 ± 3.023*** |
| TEST(Low dose-250mg/ml) | 300 | 300ns |
| TEST(High dose-500mg/ml) | 300 | 290.8 ± 9.200ns |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001; ns= non-significant; N = 5.

**Figure-8: Effect of the Formulation on Motor- coordination test using Rotarod apparatus in mice.**




Effect of the Formulation on Motor- coordination test.Values were expressed as mean ± SEM. ***P<0.001; ns= non-significant; N = 5.

## 8. 10. Effect of Kalyanakam kashayam on Apomorphine induced stereotypy in mice.

The stereotypic behavior induced by Apomorphine was assessed in mice, after 30 minutes of sample administration at doses 250 and 500 mg/kg and the results obtained are shown in table-17 and figure-9.

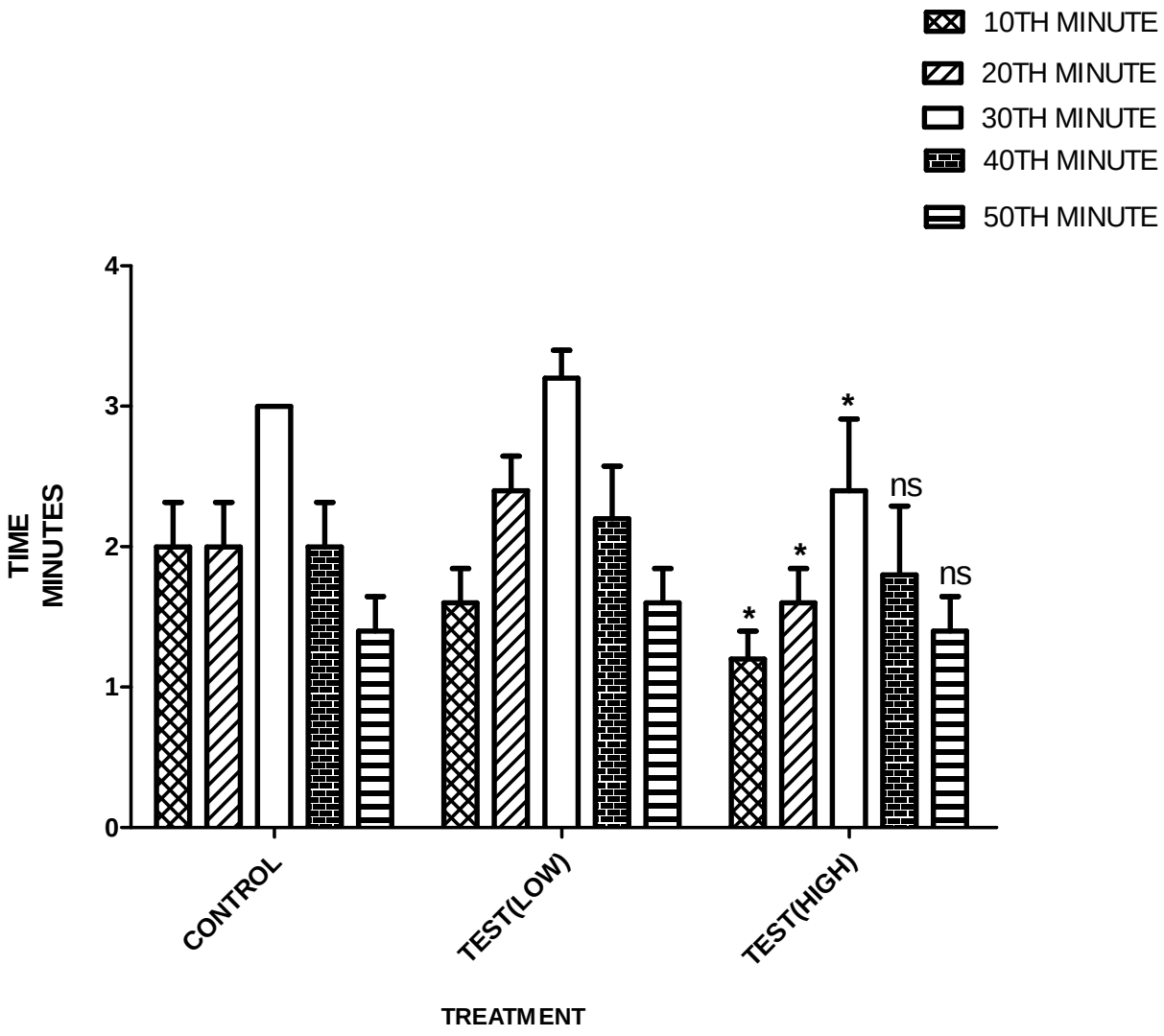
**Table-17: Effect of the Formulation on Apomorphine induced stereotypy**

| TREATMENT | SCORING FOR STEREOTYPIC BEHAVIOUR | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| CONTROL | 2 ± 0.32 | 2 ± 0.32 | 3 | 2 ± 0.32 | 1.4 ± 0.24 |
| TEST(Low dose-250mg/ml) | 1.6 ± 0.24ns | 2.4 ± 0.24ns | 3.2 ± 0.20ns | 2.2 ± 0.37ns | 1.6 ± 0.24ns |
| TEST(High dose-500mg/ml) | 1.2 ± 0.20* | 1.4 ± 0.24* | 2.3 ± 0.51* | 1.8 ± 0.49ns | 1.4 ± 0.24ns |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

*P<0.05; ns= non significant; N = 5.

**Figure-9: Effect of the Formulation on Apomorphine induced stereotypy in mice.**




Effect of the Formulation on Apomorphine induced stereotypy.Values were expressed as mean ± SEM. *P<0.05; ns= non significant; N = 5.

## 8. 11. Effect of the Formulation on Catalepsy induced by Haloperidol in mice

The Formulation at doses 250 and 500 mg/kg were administered for evaluating the cataleptic behavior of the animals placed on a horizontal bar at two different heights. The reduction in the cataleptic time was assessed. The data obtained are shown in the table-18 and figure-12.

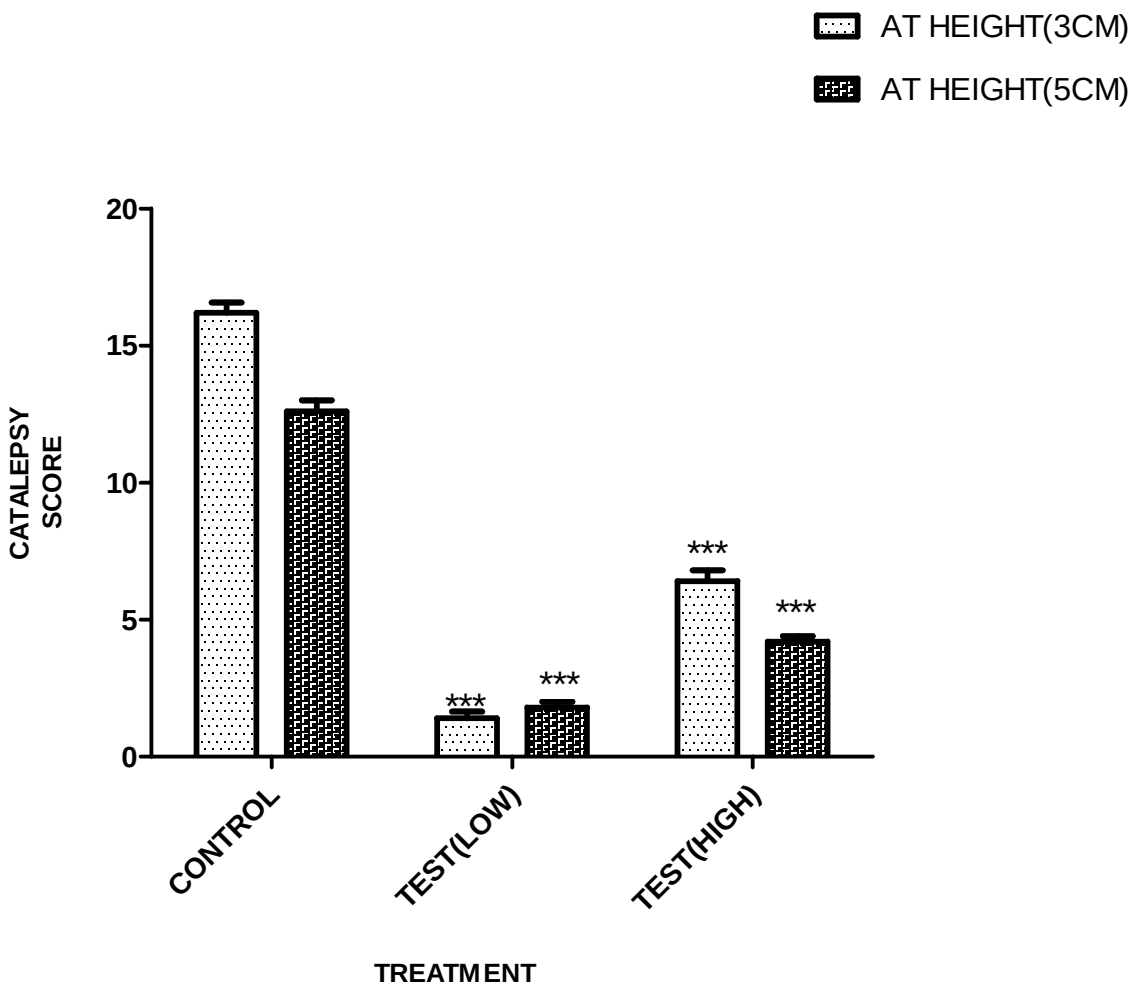
**Table-18: Effect of Kalyanakam kashayam on Haloperidol induced catalepsy**

| TREATMENT | CATALEPSY SCORE AT A HEIGHT OF; | |
|---|---|---|
| | 3CM | 5CM |
| CONTROL | 16.20 ± 0.37 | 12.60 ± 0.40 |
| TEST(Low dose-250mg/ml) | 1.4 ± 0.24*** | 1.8 ± 0.20*** |
| TEST(High dose-500mg/ml) | 6.4 ± 0.40*** | 4.2 ± 0.20*** |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

***P<0.001;N = 5.

**Figure-10: Effect of Kalyanakam kashayam on Haloperidol induced catalepsy.**




Values were expressed as mean ± SEM.***P<0.001;N = 5.

## 8. 12. Effect of the Formulation on Pentylenetetrazole induced (PTZ) induced convulsions

The effect of the formulation at doses 250 and 500 mg/kg on PTZ induced convulsions was compared with the control group and the standard group (Diazepam 4mg/kg). PTZ was administered at a dose of 80mg/kg to each animal. The observations are given in table-19 and figure-11 & 12.

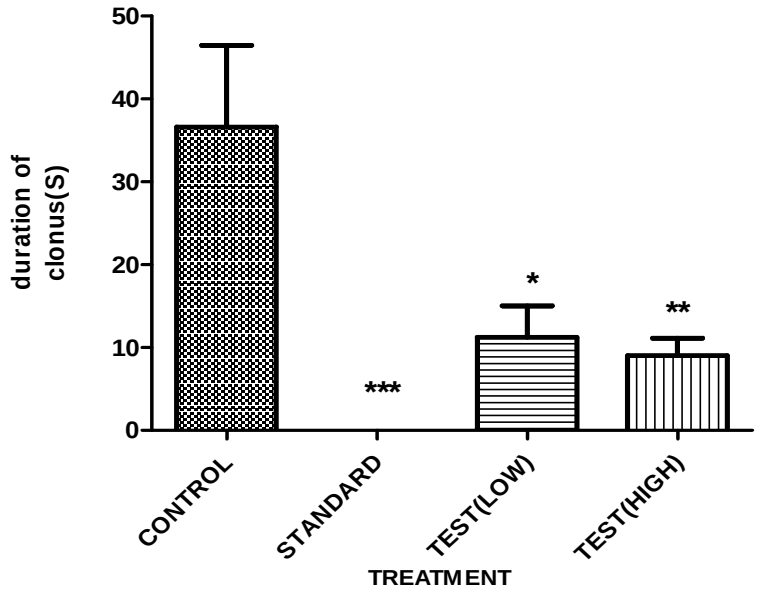
**Table-19: Effect of the Formulation on Pentylenetetrazole induced (PTZ) induced convulsions**

| **TREATMENT** | **CONVULSIONS/JERKY MOVEMENTS** | | **STATUS OF THE ANIMAL** |
|---|---|---|---|
| | ONSET(S) | DURATION(S) | |
| CONTROL + PTZ(80mg/kg) | 150 ± 19.69 | 36.60 ± 9.84 | 2 died |
| STANDARD(Diazepam 4mg/kg) + PTZ(80mg/kg) | 0*** | 0*** | All alive |
| TEST(Low dose-250mg/ml) + PTZ(80mg/kg) | 175 ± 3.76ns | 11.20 ± 3.81* | 1 died |
| TEST(High dose-500mg/ml) + PTZ(80mg/kg) | 133 ± 2.92ns | 9.00 ± 2.12** | All alive |

Values were expressed as mean ± SEM. Statistical analysis was done by one way ANOVA followed by Dunnett's test as compared to the control group.

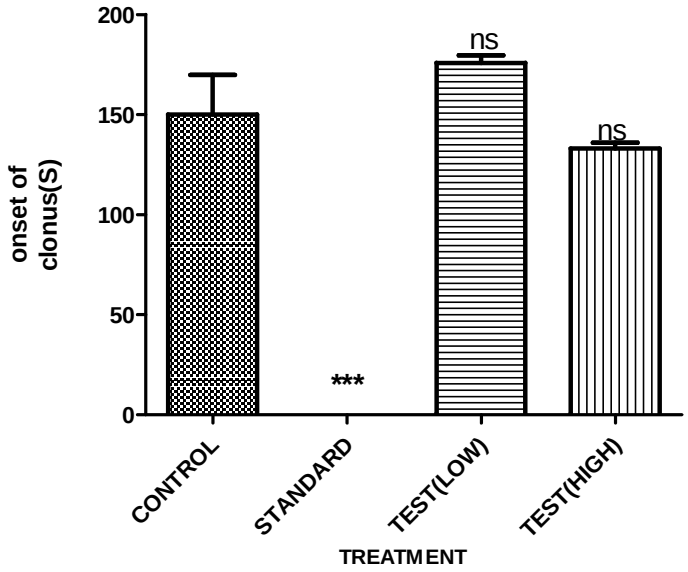
***P<0.001; **P<0.01; *P<0.05; ns= non significant;N = 5.

**Figure-11: Effect of Kalyanakam kashayam on duration of convulsions, induced by Pentylenetetrazole**




Values were expressed as mean ± SEM.***P<0.001; **P<0.01; *P<0.05; ns= non significant; N = 5.

**Figure-12: Effect of Kalyanakam kashayam on onset of convulsions, induced by Pentylenetetrazole**




Values were expressed as mean ± SEM.***P<0.001; ns= non significant; N = 5.

# 9. DISCUSSION

Advance in modern science and technology have contributed to a massive advancement in the quality of human life. However, contemporary life stresses are accountable for the surge in frequency of diversity of neurologic and psychiatric disorders like anxiety, depression, psychosis, schizophrenia, Parkinsonism, Alzheimer's disease, epilepsy etc. The drugs which are currently used for the treatment of these disorders provide symptomatic relief rather than altering the course of the disease. The Adverse effect of the drugs ranges from exasperating to hindering or potentially incurable effects. Furthermore, the overall functions and eminence of life outcome of patients still remains pitiable after treatment. Thus, for the treatment of diseases, there is a critical need to search for less toxic and more effective drugs. In this context, an increasing number of herbal products have been introduced as an alternative for treating these neurolologic and psychiatric disorders. Ayurveda in contrast to the modern western medicine considers detoxification as the prime part of treatment and believes in the fact that ailments continues cracking up again and again as far as the disease causing factors are accessible in the body. The herbal products owing to their less toxicity and adverse effects when compared with allopathic treatment have gained widespread utility for treatment of neuropsychopharmacological diseases [106] [107].

Thus Herbs and Herbal medicines have stood the test of time for their wellbeing, effectiveness, adequacy and lesser side effects. The current study deals with the inquiry of the effects of **KALYANAKAM KASHAYAM,** an Ayurvedic formulation in different animal models in the area of neuropsychopharmacology.

Herbal drugs containing radical scavengers are gaining importance in treating oxidative stress related diseases [108] [larson]. DPPH radical scavenging activity of the formulation at different concentration was compared with the standard Quercetin. Smaller $IC_{50}$ value indicates a higher antioxidant potential. The $IC_{50}$ value of the standard and test formulation was found to be 10.38µg/ml and 17.14µg/ml respectively. The higher $IC_{50}$ values of the sample indicated lesser scavenging activity of the sample when compared with the standard. The study showed the promising radical scavenging activity of the formulation due to hydrogen-donating ability of the formulation.

The ability of the formulation to donate hydrogen atoms or electrons to scavenge the radical cation was reflected by the decolourization of ABTS radical cation. The radical scavenging activity of the formulation was compared with that of the standard and the $IC_{50}$ values obtained were 0.62µg/ml and 11.29µg/ml for standard and formulation respectively.

The reducing power of the extracts may be due to the biologically active compounds in the formulation which possess potent donating abilities [109]. The reducing power of the sample was compared with the standard and the reducing power of the formulation and the standard drug was found to increase with the increase in concentration of the formulation.

Total phenolic content was determined using the Folin Ciocalteu (FC) reagent method with slight modification. Plant phenols constitute the major group of compounds that act as primary antioxidant. They can react with active oxygen radicals, such as hydroxyl radicals, superoxide anion radicals and lipid peroxyl radicals and inhibit the lipid peroxidation at an early stage. [110]. The total phenolic content of the formulation was found to be 44.29mg/g calculated as Gallic acid equivalent.

Any medicine, whether synthetic or herbal is anticipated to benefit the recipient. To validate the safety and efficacy of the same, toxicity studies are of prime importance which predicts the potential toxicity of the medicine in human beings exposed to near fatal doses. The dose and extent of toxicity of the formulation was found out by acute toxicity studies according to OECD guidelines 423 in mice with the formulation at doses 500mg/kg, 2000mg/kg and 5000mg/kg. No mortality or morbidity was found till 14 days of study. The dose fixed for current study was 250mg/ml as low dose and 500mg/kg as high dose.

The locomotor activity was assessed using the formulation and standard drug treated animals [111]. The formulation in the given doses showed noteworthy effect on locomotor activity. The formulation at a dose of 500mg/kg showed significant increase in locomotor activity than that of the formulation at low dose ie 250mg/kg. The action produced by the formulation and standard drug was comparable. The results of the study demonstrated the formulation to be an effective anti-depressant agent.

The decisive factor for sleep was the loss of righting reflex and the period between the loss and revival of righting reflex was used as the indication of hypnotic effect

[112]. The formulation at a dose of 500mg/kg showed a promising increase in the duration of sleep when compared with the formulation at a dose of 250mg/kg.

Animals when forced to swim in a narrow space, from which it cannot escape, initially adopts a period of vigorous activity followed by an immobile posture, with movements necessary to keep their heads above water. The immobility posture was described as a sign of behavioral despair. Current studies have proved that, clinically active anti-depressants could greatly reduce the immobility time induced in the animal [113]. The formulation at doses 250mg/kg and 500 mg/kg showed mild reduction in immobility time observed, when compared with the standard drug (Mephentramine 30mg/kg) which showed a significant reduction in the duration of immobility in mice.

EPM model is an etiologically convincing animal model of anxiety since it uses natural stimuli ie apprehension of new open space and fear of balancing on a comparatively lifted and slender platform (that could provoke anxiety in humans). The number of entries into the open arms and the time spent in open arms is increased by an anxiolytic agent [114]. The formulation at both doses did not affect the number of entries to both the arms. Standard drug diazepam at a dose of 5mg/kg increased the time spent and number of entries made by the animal in open arms. Formulation (low dose) showed moderate increase in the time spent in open arm as compared to the control group, whereas formulation (high dose) significantly increased the time spent by the animal in open arm as compared to the control group.

Hole board apparatus was considered as a promising animal model for evaluating the anxiolytic activity of drugs. The reduction in the number of nose poking was considered as an indicator of anxiolytic activity. The formulation at a concentration of 500mg/kg showed significant reduction in the number of nose poking made by the animal. The reduction in nose poking was comparable with that of the standard drug diazepam (5mg/kg). Formulation at a dose of 500mg/kg showed better anxiolytic activity when compared with the formulation at a dose of 250mg/kg.

The formulation at doses 250mg/kg and 500mg/kg and standard drug Diazepam 5mg/kg were used for evaluating the motor coordination in mice. The formulation at both the doses did not show any significant changes in the fall of time suggesting that the

formulation did not induce disturbances in motor coordination at the given doses, whereas the standard drug produced a significant reduction in fall off time. The results suggested that Kalyanakam kashayam did not show any signs of neurotoxicity.

The utmost intensity of stereotypy induced by Apomorphine was observed at 30 minutes. Stereotypy behaviour induced by Apomorphine was un-effected after the administration of the formulation at a dose of 250mg/kg. Whereas, the formulation at a dose of 500mg/kg produced a mild drop in the stereotypic behaviour induced by Apomorphine. The results recommended that the formulation at a dose of 500mg/kg is an impending atypical antipsychotic against schizophrenia.

The tranquillizer activity and the motor effects of drugs (those related to extra-pyramidal system) were evaluated by using this test. Conventional antipsychotic drugs well known for its foremost adverse effects like catalepsy, which forms a vital part concerned in the discovery and development of the antipsychotic drugs [115]. The Formulation at doses 250 and 500 mg/kg were administered for evaluating the cataleptic behavior of the animals placed on a horizontal bar at two different heights 3cm and 5cm. The formulation at both the doses showed significant reduction in the cataleptic time when compared to the control group animals. The formulation at a dose of 500mg/kg was found to produce a significant reduction in the cataleptic score when compared with the score produced by the formulation at a lower dose (250mg/kg).

The PTZ-induced seizures are comparable to the symptoms observed in the absence seizures (petit mal epilepsy), and drugs useful in the treatment of this type of epilepsy also suppress PTZ-induced seizures [116]. The antiepileptic activity of the formulation was evaluated in mice at two different doses, ie 250mg/kg (low dose) and 500 mg/kg (high dose), which was compared with that of the standard drug diazepam (4mg/kg). The standard drug produced promising anti-convulsant activity at the given dose, whereas the formulation showed better anti-convulsant activity at a dose of 500mg/kg when compared with the formulation at a dose of 250mg/kg. Formulation treated did not show any significant effect on the onset of convulsions. The animals treated with formulation and standard drugs were protected from death.

# 10. CONCLUSION

The current study reveals that Kalyanakam Kashayam, an Ayurvedic polyherbal formulation possesses significant neuropsychopharmacological activity. The activities were evaluated using various animal models.

The formulation showed significant *in vitro* anti-oxidant by ceasing the actions of free radicals. The formulation was studied for its anti-anxiety effect using elevated plus maze model and hole board test. Sleeping time assessment, forced swim test and spontaneous locomotor activity assessment were the models selected for evaluating the anti-depressant activity. The other animal models used were rotarod test for evaluating neurotoxicity, apomorphine induced stereotypy, haloperidol induced catalepsy and PTZ induced convulsions in mice.

The results suggested that the formulation Kalyanakam Kashayam produced significant potetiation of sleeping time induced by pentobarbitone; significant increase in locomotor activity; mild reduction in immobility time in forced swim test; significant increase in the time spent in open arm without effecting the number of entries to both the arms in elevated plus maze model, significant reduction in the number of nose poking in hole board test, significant reduction in cataleptic time induced by Haloperidol. The sample was found to be a better antipsychotic agent for the treatment of schizophrenia and is a non-neurotoxic drug. The anti-convulsant activity of the formulation was revealed in PTZ induced seizure models.

Thus from the above reports it was concluded that the Ayurvedic formulation Kalyanakam Kashayam showed anti-oxidant activity and promising nootropic activity in Swiss Albino mice.

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